THE SIGNIFICANCE OF THE ISOLATION OF CORYNEBACTERIUM SPECIES FROM URINE OF PATIENTS AT BARAGWANATH HOSPITAL.

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ABSTRACT

The purpose of this study was firstly to determine whether corynebacteria, when isolated from urine specimens, represent significant pathogens and secondly to attempt to increase the isolation rate of Corynebacterium urealyticum, a known urinary pathogen by culturing urine specimens on a selective medium. An attempt was made to identify all isolates of corynebacteria to the species level and antimicrobial susceptibility testing was performed on all organisms.

In the first part of the study, forty-four (0,56 per cent) of 7,912 urine specimens yielded $>10^4$ corynebacteria per ml of urine. Three control groups were selected with forty-four patients in each. The groups included patients from whose urine Escherichia coli, Staphylococcus epidermidis and no organisms were isolated respectively. Although the presence of pyuria did not differ significantly between the study group and the Escherichia coli group, the presence of symptoms of urinary tract infection was significantly higher in the Escherichia coli group than in the study group and based on this difference, corynebacteria as a group could not be implicated as urinary tract pathogens in this study. Thirty-four of forty-four isolates of corynebacteria were identified to the species level. Five isolates were identified as Corynebacterium urealyticum. All the patients in this group demonstrated pyuria, haematuria and symptoms of urinary tract infection, and four patients had a history of urological manipulation and underlying disease, confirming the role of this organism as a urinary pathogen in the compromised host.

Antimicrobial susceptibility testing of corynebacteria other than Corynebacterium urealyticum, revealed universal susceptibility to vancomycin and high susceptibility rates to norfloxacin, aminoglycosides and the cephalosporins excluding ceftazidime. The five isolates of Corynebacterium urealyticum demonstrated universal susceptibility only to vancomycin.

A second study was designed to investigate the use of a selective medium to increase the yield of Corynebacterium urealyticum. 1,287 urine specimens yielded sixteen (1,2 per cent) isolates of Corynebacterium urealyticum on the selective medium. Eighty-eight per cent of these specimens yielded another pathogen concomitantly, suggesting that the isolates of Corynebacterium urealyticum, being common groin colonizers in hospitalized patients, may have been contaminants. The use of selective media in hospitals where urine specimens are not collected according to strict aseptic techniques, is therefore not recommended since it appears to increase the yield of contaminants. Selective media could possibly be used where urinary pH is alkaline and triple phosphate crystals are present, since these characteristics commonly feature in Corynebacterium urealyticum infection.

Antimicrobial susceptibility testing was performed on the sixteen isolates of Corynebacterium urealyticum and the four isolates of Corynebacterium jeikeium which grew on the selective media. Many isolates were multiresistant and vancomycin was the only antimicrobial to which all isolates were susceptible.

DECLARATION

I declare that this dissertation is my own unaided work. It is being submitted for the degree of Master of Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other university.

D D Walkden

Deborah Dianne Walkden

This 30th day of March, 1993.

The methods employed in this study were approved by the committee for research on human subjects of the University of the Witwatersrand.

Ref: R14/49, 4 March 1991.

I dedicate this dissertation to my two small daughters, Erin and Robyn, without whose help the work would have been completed in half the time.

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PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS DISSERTATION.

1. Walkden D, Klugman KP, Vally S, Naidoo P. (1993). Urinary tract infection with Corynebacterium urealyticum in South Africa. Eur J Clin Microbiol Infect Dis (in press)

2. Poster presentation. The significance of the isolation of corynebacteria from urine specimens of patients at Baragwanath hospital. Joint Congress of the Infectious Diseases Society of Southern Africa and The Sexually Transmitted Diseases Society of Southern Africa, September 1991.

3. Poster presentation. Urinary tract infection with Corynebacterium Group D2 at Baragwanath hospital. Federation of South African Societies of Pathology 32nd Annual Congress, June 1992.

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1. INTRODUCTION AND OBJECTIVES OF THE STUDY

The term "coryneform" is traditionally applied to any Gram-positive, non-sporing, pleomorphic, non-branching bacillus encountered in the clinical microbiology laboratory. This description covers many genera of bacteria, including Corynebacteria, Actinomyces, Arachnia, Bifidobacteria, Erisipelothrix, Listeria, Propionibacteria, Rhodococcus and Rothia. This diversity of genera renders the identification to species level of most of these organisms, a formidable task.

In addition, accurate taxonomic classification of many of these genera requires such procedures as identification of cell wall components, including mycolic acids and fatty acids by gas-liquid chromatography and high-performance liquid chromatography (Corina, 1980; Athalye, 1984 and 1985; Butler, 1986; Bernard, 1991;).

Sequencing of rRNA (Park, 1987), mass spectrometry of isoprenoid quinones (Collins, 1979) and DNA base composition and nucleic acid hybridization have all been used to establish taxonomic relatedness of strains (Coyle, 1990). Since most of these procedures are beyond the scope of the average clinical laboratory and since many of these isolates are common skin and environmental commensals, in many cases only the most pathogenic isolates are identified to the species level.

In this study, all organisms demonstrating typical morphology on Gram stain and producing catalase, were defined as corynebacteria.

Corynebacteria constitute a significant component of resident skin flora (Marples, 1969) and these organisms, normally of low pathogenicity may cause invasive disease, especially in the modern era of iatrogenic immunosuppression and invasive procedures. These organisms, when cultured from urine specimens, are usually dismissed as contaminants, however a study undertaken by Maskell (1990) implicated corynebacteria as causative agents in urinary tract infections.

Corynebacterium urealyticum, a recently discovered urinary pathogen in Europe has been documented only once in the context of urinary tract infection in South Africa (Stewart, 1993) and no data exist on the role of this organism as a pathogen in a third world population.

The purposes of this study were thus as follows:

1. To investigate the clinical significance of the isolation of corynebacteria from urine specimens of patients at Baragwanath hospital.

2. To attempt to identify the isolates and determine their antimicrobial susceptibilities.

3. To attempt to increase the isolation rate of Corynebacterium urealyticum by the use of a selective medium.

4. To determine the incidence of infection with, and the pathogenic role played by Corynebacterium urealyticum in patients at Baragwanath hospital.

2. LITERATURE REVIEW

2.1 Corynebacteria as urinary pathogens

a. Corynebacterium urealyticum

In 1985 a role for Corynebacterium urealyticum (previously known as Corynebacterium Group D2) (Pitcher, 1992) as a urinary pathogen was suggested. A report of four cases by Soriano et al (1985), implicated this organism in a condition known as alkaline encrusted cystitis. This condition was first described by Francois in 1914 as an ulcerative condition in which ammonium magnesium phosphate crystals were deposited on the bladder mucosa (Cited by Soriano (1985)). In 1925, Hager and Magath attributed this disorder to infection by urea splitting organisms in a bladder already damaged by inflammation or tumour, providing vesicular ground. Organisms implicated in this condition prior to the isolation of Corynebacterium urealyticum include streptococci, staphylococci, Proteus species and other enterobacteriaeceae (Hager, 1925; Randall, 1937; Coyle, 1990).

Further work by the Spanish group and others (Soriano, 1986; Aguado, 1987; Schoch, 1987; Nadal, 1988; Sofras, 1988; Soriano. 1988a; Ronci Koenig, 1990; Soriano, 1990) elucidated the clinical and laboratory features of infection with this corynebacterium, as well as predisposing factors contributing toward infection.

i. Clinical features

The clinical features of infection with Corynebacterium urealyticum vary. Thirty-eight per cent of patients in one series (Soriano, 1990) and 16 per cent in another were assymptomatic (Aguado, 1987). Men were found to manifest symptoms more often than women (Soriano, 1990).

Symptomatic patients presented with acute cystitis, chronic cystitis or pyelonephritis (Aguado, 1987; Nadal, 1988; Soriano, 1988; Soriano, 1990). In cases of acute cystitis, dysuria, frequency, urgency and suprapubic pain occurred as short term symptoms. Patients with chronic cystitis were more likely to have mucosal encrustations, pass gritty urine with a strong ammonia odour and relapse after treatment. Pyelonephritis occurred significantly more often in patients with underlying disease and manifested with dysuria, fever, flank pain and malaise.

ii. Predisposing factors

In several series of patients, various factors predisposing to infection were consistently found.

a. Immunosuppression, including steroid and cytostatic therapy, haematologic malignancy, solid tumours and AIDS (Aguado, 1987; Ronci-Koenig, 1990; Soriano, 1990).

b. Chronic debilitating disease including diabetes mellitus, rheumatoid arthritis, hepatic cirrhosis, chronic bronchitis and neurological disease (Ronci-Koenig, 1990; Soriano, 1990; Ohl, 1992).

c. Antibiotic usage during the two months prior to the isolation of Corynebacterium urealyticum (Aguado, 1987; Nadal, 1988; Ronci- Koenig, 1990; Soriano, 1990).

d. Underlying urological disease,

- prostatic hypertrophy (Aguado, 1987).
- post renal transplantation (Aguado, 1987; Soriano, 1990; Morales, 1992).
- megacalycosis (Nadal, 1988).
- previous renal or bladder pathology or a urinary tract infection due to other organisms in the week preceding the isolation of Corynebacterium urealyticum

(Aguado, 1987; Nadal, 1988; Soriano, 1990).

- radiation cystitis (Aguado, 1987).
- renal cyst (Ohl, 1992).

e. Prior urologic manipulations including catheterization (Sofras, 1988), transurethral resection and cystostomy (Soriano, 1990).

iii. Characteristics of urine in patients with Corynebacterium urealyticum bacteriuria

Urine from patients with Corynebacterium urealyticum bacteriuria usually has an alkaline pH and microscopically demonstrates pyuria, haematuria and struvite crystals (Soriano, 1990; Morales, 1992; Ohl, 1992), and an odour of ammonia (Soriano, 1986; Aguado, 1987; Nadal, 1988). If cystoscopy is performed, encrusted cystitis with calculi and underlying muscular fibrosis may be seen (Sofras, 1988; Soriano, 1990).

iv. Epidemiology

Corynebacterium urealyticum may commonly be isolated from the skin of hospitalized patients, especially females. The prevalence of colonization increases with the length of hospitalization. In one series, colonization rates for hospitalized females ranged from 39,6 per cent to 47,1 per cent and for males from 8,5 per cent to 26,5 per cent (Soriano, 1988b). The area most frequently colonized is the groin. It is possible that the organism invades the urinary tract from the skin in predisposed patients.

v. Pathogenesis and virulence factors

In vitro attempts at determining the pathogenesis of alkaline encrusted cystitis were carried out by Hager and Magath in 1925, in which they inoculated a urealytic organism, 'Salmonella ammoniae', into guinea pigs bladders. The organism only proved pathogenic in bladders in which an underlying chemical cystitis had been induced by prior instillation of salicylic acid and not in normal bladders. The pathology consisted of ulcerations with the deposition of hard gritty material on the affected areas and in some cases stone formation. The authors postulated that the rapid production of ammonia from urea by this organism precipitated alkaline inorganic salts.

In 1986, Soriano et al proved that Corynebacterium urealyticum inoculated into human urine, caused formation of ammonium magnesium phosphate (struvite) crystals. The pH and the ammonium concentration of the urine had increased after twenty-four hours. Escherichia coli growing in human urine did not cause crystal formation and the ammonium concentration and pH of the urine remained unchanged. In addition, Corynebacterium urealyticum inoculated onto zinc discs which were subsequently inserted into rat bladders, induced stone formation in sixteen out of twenty-four animals.

It has therefore been postulated, that hydrolysis of urea by bacterial ureases causes hyperammonuria and alkalinization of the urine, inducing hypersaturation with struvite ($Mg\ NH_4\ PO_4\ 6H_2O$) and calcium phosphate with crystal formation (Griffith, 1982; Soriano, 1986).

In an attempt to define the virulence factors of Corynebacterium urealyticum, Marty et al (1991) examined sixty-one strains for their ability to adhere to human uroepithelial cells and to agglutinate human and guinea pig erythrocytes. The

presence or absence of piliation was assessed by transmission electron microscopy. Forty-six of the strains examined were isolated from bacteriuric patients and fifteen from the skin of healthy patients.

There was no statistically significant difference between the two groups with respect to adherence, haemagglutination or both activities and although piliation was present only in adherent / haemagglutinating isolates, it was not present in all such isolates. This indicates that the presence of pili and the ability of strains to adhere or haemagglutinate do not serve as markers of virulence in Corynebacterium urealyticum.

vi. Antimicrobial susceptibility and treatment

Corynebacterium urealyticum is highly resistant to many antimicrobials. The mechanism of resistance may be due to changes in the permeability of the cell wall as has been described in Corynebacterium jeikeium (Stamm, 1979). These changes may be due to selective antibiotic pressure on skin colonizers, however Fernandez-Roblas et al, (1987) noted no significant difference in antimicrobial susceptibility between clinically significant isolates and colonizing isolates.

Strains of Corynebacterium urealyticum, tested for antimicrobial susceptibility by various groups, indicate an almost universal susceptibility to vancomycin and teicoplanin, with minimum inhibitory concentrations (MIC) being lower at pH 7,4 than at pH 8,5 (Santamaria, 1985; Fernandez Roblas, 1987; Soriano, 1987; Philippon, 1990; Garcia Rodriguez, 1991).

The quinolones, ciprofloxacin and ofloxacin, are active against many isolates, while norfloxacin is less active (Santamaria, 1985). In 1985 and 1987, all strains tested by Santamaria and Soriano, respectively, were susceptible to norfloxacin. A 1991 survey by Garcia Rodriguez documented resistance of Corynebacterium urealyticum to the quinolones varying from 53 per cent resistance to ofloxacin to

90 per cent resistance to enoxacin. Although resistance to quinolones seems to be increasing, norfloxacin may still achieve concentrations in the urine above the MIC for most strains of Corynebacterium urealyticum and is therefore useful for treating urinary tract infections (Santamaria, 1985; Soriano, 1987). Norfloxacin is also least affected by the alkaline pH of the urine (Soriano, 1987).

Other antibiotics which have been tested include penicillins, aminoglycosides, cephalosporins, macrolides, tetracyclines, novobiocin, rifampicin, fucidic acid, sulphamethxazole / trimethoprim, nitrofurantoin and nalidixic acid (Santamaria, 1985; Fernandez-Roblas, 1987; Soriano, 1987; Philippon, 1990). The susceptibility of strains varied in the different series, generally demonstrating more susceptibility in earlier studies than in the study published by Garcia-Rodriguez in 1991, in which the only reliably active drugs were the glycopeptides.

Philippon (1990) found all strains highly sensitive to fusidic acid (mean MIC 0.047 mg/ml) and the streptogramin, pristinamycin (mean MIC 0.048 mg/ml). A few strains were sensitive to the macrolides, rifampicin, tetracycline and gentamicin, although in Soriano's series (1987) erythromycin and tetracycline were bacteriostatic only.

The in vitro activity of the streptogramin group of antimicrobials against Corynebacterium urealyticum was also investigated by Arora (1989). They demonstrated good activity against all isolates, with MIC's ranging from 0,5 -1 mg/ml, for both pristinamycin and virginiamycin. Clinical evaluation of the efficacy of these antimicrobials in the treatment of urinary tract infections has not been undertaken.

The use of acetohydroxamic acid, a urease inhibitor , together with antimicrobial agents in the treatment of Corynebacterium urealyticum urinary tract infections, shows no synergy when combined with norfloxacin or any antimicrobial activity alone (Soriano, 1987). This agent could however be useful in creating a better pH

milieu for the antimicrobials to act, since the alkaline urine and struvite crystals create an unfavourable environment for the action of most antibiotics (Santamaria, 1985).

Various antibiotic regimens have been used to treat patients with Corynebacterium urealyticum urinary tract infection. In one series (Soriano, 1990), 27 per cent of twenty-two patients treated with an appropriate antibiotic relapsed due to in vivo development of resistance during therapy. The antibiotics administered were tetracycline, rifampicin, erythromycin, josamycin, amikacin and gentamicin.

In a recent study (Soriano, 1991), a rat model was used to assess the effectiveness of ofloxacin, ciprofloxacin in low and high doses and teicoplanin against the development of encrustations on zinc disks implanted into bladders after infection with Corynebacterium urealyticum. The results of this experiment demonstrated the superiority of teicoplanin and high dose ciprofloxacin over the other regimens in decreasing both the mortality rates of the rats and the extent of encrustation on the zinc discs. This evidence, together with the increasing resistance of Corynebacterium urealyticum to the quinolones (Soriano, 1989), suggests that the glycopeptide antimicrobials, vancomycin and teicoplanin are the treatment of choice for Corynebacterium urealyticum induced cystitis.

In addition to antimicrobial therapy with or without acetohydroxamic acid, patients in whom encrustations have developed, frequently require resection of the latter before microbiological cure can be achieved (Soriano, 1990).

vii. Corynebacterium urealyticum in other infections.

Although Corynebacterium urealyticum has often been isolated from specimens other than urine, the significance of its isolation has been questioned. Being a skin colonizer, the aetiological role of Corynebacterium urealyticum in wound and other infections may be difficult to assess. In one series (Van Bosterhout, 1987), seven

strains isolated from blood cultures were regarded by the authors either as contaminants, or as producing a transient bacteraemia. The same authors did however, isolate Corynebacterium urealyticum from the peritoneal fluid of a patient undergoing chronic ambulatory peritoneal dialysis. The patient had symptomatic peritonitis.

Bacteraemia may occur in patients with underlying urinary tract infection. Agnado (1987) isolated Corynebacterium urealyticum from the blood of four of their patients concomitantly with isolation of the organism from the urine. Marshall (1987) reported a case where the organism was isolated on three occasions from the blood of a renal transplant recipient. The rejected kidney was found to be necrotic on removal. The patient recovered despite treatment with cefotaxime to which the MIC for the isolate was >32 mg/ml, suggesting that the bacteraemia had been self limiting.

Corynebacterium urealyticum was initially implicated in human infection other than urinary tract infection in 1979 (Jacobs), when the organism was isolated from a tracheal aspirate in an elderly debilitated patient with pneumonia. Unlike most Corynebacterium urealyticum strains this isolate was highly sensitive to penicillin.

In 1991, Corynebacterium urealyticum was implicated in a case of aortic and mitral valve endocarditis in an intravenous drug abuser (Ena, 1991), being cultured from six blood specimens and both valves. The most likely portal of entry in this case was the skin via an injection site.

Another case of proven infection due to Corynebacterium urealyticum was that of osteomyelitis in an elderly male following a compound fracture of the femur. Therapy with vancomycin and subsequently teicoplanin was successful in this patient (Chomarat, 1991).

b. Corynebacterium pseudodiphtheriticum

Corynebacterium pseudodiphtheriticum (hofmanii) is considered normal human pharyngeal flora, rarely being implicated in infective endocarditis (Brown, 1990). In 1982, Nathan et al, reported a case of necrotic infection involving the entire urinary tract in an immunocompromized patient post renal transplantation. Culture of the explanted kidney revealed a pure growth of Corynebacterium pseudodiphtheriticum. This organism had previously been isolated from the urine of this patient.

c. Corynebacterium group E

A case of pyelonephritis and septicaemia due to infection by Corynebacterium group E or aerotolerant Bifidobacterium adolescentis was reported in 1980 (Guillard). This patient had a history of injury to the urinary tract followed by repeated urethral dilatations and a previous episode of Escherichia coli pyelonephritis. The organism was isolated in pure culture of $>10^5$ organisms per ml, from two separate midstream clean catch specimens.

d. Corynebacterium aquaticum

Corynebacterium aquaticum is a normal inhabitant of fresh water. The organism has rarely been implicated in human disease but has been described as the causative agent in a case of neonatal urinary tract infection, (Tendler, 1989) and a case of neonatal meningitis (Beckwith, 1980). The patient with the urinary tract infection, was a healthy eight day neonate, with no evidence of urinary tract abnormality, who presented with vomiting and diarrhoea. Culture of the urine revealed Corynebacterium aquaticum in pure growth. The child responded to the

appropriate antibiotic therapy. As a neonatal pathogen, Corynebacterium aquaticum may cause confusion, since morphologically and biochemically it resembles Listeria monocytogenes.

e. Corynebacterium genitalium

In 1977, Furness et al isolated Corynebacterium genitalium from fifty-two non-gonococcal urethritis patients or their consorts and from no controls. This evidence led them to implicate the organism as a cause of non-gonococcal urethritis but no other work has corroborated these results.

f. Corynebacterium Group F1

A recent case report (Digenis, 1991), implicated this organism in a urinary tract infection in a forty year old male. It is interesting to note that this species of corynebacteria also produces urease and in this case, caused the formation of struvite calculi. The patient ceased to pass stones within three days of the appropriate treatment being administered.

g. Other corynebacteria

Corynebacteria (not further identified) were isolated from 182 urine specimens over a six month period in 1988 (Maskell). These isolates were considered to be significant if the patients had symptoms or pyuria, the corynebacteria were isolated in pure or almost pure culture and no other pathogens were isolated. These corynebacteria represented 2 per cent of all urinary isolates during that period. In a control group of 176 patients without symptoms of urinary tract infection, corynebacteria were isolated from only seven specimens of urine, one in pure

culture and six in mixed culture. These results led the authors to conclude that corynebacteria other than Corynebacterium urealyticum may assume a significant role in urinary tract infections.

2.2 Other corynebacteria in disease

Since corynebacteria are common commensal flora of humans, animals and the environment, they often appear in cultures of clinical specimens and are dismissed as contaminants. There are however several species which are documented causes of human infection. These may be divided into four groups:-

- Known human pathogens
- Human opportunists
- Animal pathogens which occasionally infect humans.
- Environmental commensals which occasionally infect humans.

a. Known human pathogens

i. Corynebacterium diphtheriae

Corynebacterium diphtheriae, the type species of the genus Corynebacterium was responsible for major epidemics which decimated large numbers of people as far back as medical history is recorded. One such epidemic occurred in New England and Europe in the early eighteenth century, lasting five years. Of the total population of New England 2,5 per cent died during this time, most being children. Thereafter similar epidemics occurred at approximately twenty-five year intervals throughout the eighteenth and nineteenth centuries (English, 1985).

The disease was so prevalent that in index medicus in 1892, nearly 1 per cent of all articles listed, concerned diphtheria (English, 1985).

The year 1884 heralded the discovery of the diphtheria bacillus by Friedrich Loeffler (as cited by English, 1985) and in 1895 antitoxin became available. Initial clinical trials of immunization using a toxoid developed by Gaston Roman began in 1924 and reduced fatalities among the immunized to nearly zero (English, 1985).

Due largely to immunization, "Diphtheria's clinical history ended 50 years ago" (English, 1985), the disease being seen mainly in the unimmunized. An in depth discussion of diptheria is beyond the scope of this dissertation.

ii. Arcanobacterium (Corynebacterium) haemolyticum

Arcanobacterium (Corynebacterium) haemolyticum is an unusual cause of non-streptococcal exudative pharyngitis which may be accompanied by a scarlatiniform rash. This infection occurs predominantly in young adults (Ryan, 1972; Miller, 1986; Selander, 1986). Other reported infections are rare and include post traumatic ankle joint infection (Hoosen, 1991) and septicaemia (Goudswaard, 1988).

iii. Corynebacterium minutissimum

Corynebacterium minutissimum is believed to be the causative agent of erythrasma, a chronic skin infection. This organism has been implicated rarely in invasive infections including bacteraemia in a patient with chronic myeloid leukaemia (Guarderas, 1986) and in a chronic breast abscess (Berger, 1984).

iv. Corynebacterium tenuis

This species is believed to incorporate a group of coryneforms which are implicated in Trichomycosis axillaris, a disease of axillary hairs in which nodules and sheaths coat the hair shafts (Coyle, 1990).

b. Human opportunists

i. Corynebacterium jeikeium

In 1976, Hande et al reviewed four cases of infection due to a multiresistant corynebacterium species. The following year Pearson et al (1977) described twelve oncology patients with sepsis due to a biochemically similar organism. These organisms were later named "group JK" and characterised by Riley et al, in 1979. Since then numerous reports have appeared in the literature documenting infection caused by "Group JK" or Corynebacterium jeikeium as it is now known.

Corynebacterium jeikeium forms part of normal skin flora, particularly in the axillary, inguinal and rectal sites. The prevalence of colonization is increased in hospitalized patients (Stamm, 1979; Gill, 1981; Larson, 1986).

Septicaemia occurs mainly in immunocompromized patients, particularly those with haematological malignancies, granulocytopaenia and most recently AIDS. Other predisposing conditions include prolonged hospitalization, breaks in the integument, the presence of prosthetic devices and prolonged antibiotic therapy, since many isolates are multiresistant (Hande, 1976; Pearson, 1977; Stamm, 1979; Gill, 1981; Hoffmann, 1983; Quin, 1984; Guarino, 1987; Telander, 1988; Spach, 1991).

Clinical syndromes described include bacteraemia, endocarditis on prosthetic as well as abnormal native valves, pneumonia, peritonitis in a patient on chronic ambulatory peritoneal dialysis, meningitis, pyelonephritis, infection of a ventriculo-atrial shunt and abscesses, wound and intravascular catheter site infections (Hande, 1976; Pearson, 1977; Stamm, 1979; Gronemeyer, 1980; Gill, 1981; Plerard, 1983; Quin, 1984; Heltberg, 1986; Guarino, 1987; Telander, 1988; Mc Naughton, 1988; Spach, 1991).

ii. Corynebacterium pseudodiphtheriticum

Corynebacterium pseudodiphtheriticum, a commensal of the nasopharynx, has been implicated mainly in cases of endocarditis on both prosthetic and native valves (as cited in Coyle 1990). Other cases reported include pneumonia (Miller, 1986), suppurative lymphadenitis (La Rocco, 1987) and necrotizing tracheitis in a diabetic (Colt, 1991).

iii. Corynebacterium xerosis

This species is regarded as a commensal of mucocutaneous sites. Infections have been recorded in both immunocompromized and immunocompetent hosts. Reported cases include septic arthritis (Valenstein, 1988), vertebral osteomyelitis (Krish, 1989), pneumonia, bacteraemia and wound infection (Porschen, 1977).

iv. Corynebacterium striatum

Corynebacterium striatum, normal flora of the anterior nares and skin has been implicated rarely in pulmonary infections in immunocompromized patients (Barr, 1986; Coyle, 1990).

c. Animal pathogens which may infect humans

i. Actinomyces (Corynebacterium) pyogenes

Actinomyces (Corynebacterium) pyogenes, a common cause of bovine mastitis, has been implicated in leg ulcers, abscesses, intraabdominal infection, bacteraemia and cystitis in humans (as cited in Coyle 1990).

ii. Rhodococcus (Corynebacterium) equi

This organism, a well known pathogen for swine, cows and horses has been described mainly in cases of pulmonary infection in immunocompromized patients (van Bets, 1983). With the advent of the AIDS epidemic, Rhodococcus equi is assuming increasing significance as an opportunist in these patients (Prescot, 1991; Harvey, 1991).

iii. Corynebacterium ulcerans

Corynebacterium ulcerans is a pathogen of domestic animals, chiefly livestock, causing mastitis. Most of the human cases described have been that of throat infection with or without a diphtheria-like illness, since the organism may elaborate diphtheria toxin (Lipsky, 1982).

iv. Corynebacterium pseudotuberculosis

Corynebacterium pseudotuberculosis causes suppurative lymphadenitis in domestic animals. Human infection is rare, but a case of lymphadenitis has been reported (Lopez, 1966).

v. Corynebacterium bovis

Corynebacterium bovis, a cause of bovine mastitis, has been implicated in human endocarditis, meningitis and ventriculojugular shunt nephritis (Bolton, 1975; Vale, 1977).

3. MATERIALS AND METHODS

This study was conducted at Baragwanath Hospital and the Baragwanath Hospital branch of the South African Institute for Medical Research microbiology laboratory.

All urine specimens received at the laboratory between 15 January 1991 and 15 June 1991, which had been collected within the previous four hours, were processed in the following way:

The presence of nitrites, protein and bilirubin, the specific gravity and pH were determined with multistix reagent strips (Ames, Bayer-Miles, Slough, United Kingdom). Erythrocyte and leucocyte counts were performed using an improved Neubauer counting chamber (Baker, 1975). The sedimented urine was examined microscopically for the presence of parasite ova, epithelial cells and Trichomonas vaginalis and the presence and nature of casts and crystals.

Using a calibrated wire loop, 0,001 ml of urine was inoculated onto one of each of a 5 per cent horse blood agar plate, (Oxoid Columbia agar base, Oxoid, Basingstoke, United Kingdom) and a Mac Conkey agar plate (Oxoid). The inoculum was streaked out for single colonies and the plates were incubated at 37^0 C under 5 per cent CO_2 for twenty-four hours, after which time colonies were counted, each colony representing one thousand organisms. All urine specimens from patients older than fifteen years, from which $> 10^4$ colony forming units per millilitre (cfu/ml) corynebacteria were isolated, either in pure growth, or as the predominant organism on a plate with only one other colony type, were included in the study.

The number of colonies was chosen as $> 10^4$ per ml because it has been shown that significant urinary tract infection can occur in the presence of less than the

traditional 10^5 cfu/ml (Maskell, 1989). This may apply particularly in the case of more fastidious bacteria such as streptococci, Gardnerella vaginalis, Haemophilus influenzae and corynebacteria.

For each study case, three control urine specimens were selected as follows:

a. The first urine specimen following the study case, with a pure growth of Escherichia coli at $> 10^4$ cfu/ml (hereafter referred to as the E coli control), was selected as the first control.

b. The first specimen following the study case, from which no organisms were cultured was taken as the second control (hereafter referred to as the "no growth" control).

c. The first urine specimen following the study case, from which Staphylococcus epidermidis was cultured at $> 10^4$ cfu/ml (hereafter referred to as the S epidermidis control) was selected as the third control.

An attempt to identify all isolates of corynebacteria was made using the following biochemical criteria.

Fermentation of glucose, sucrose, maltose, mannitol and xylose was carried out in cysteine trypticase soy base agar (BBL Microbiology Systems, Cockeysville, USA) to which 1 per cent of the carbohydrate had been added. Tests were inspected after forty-eight and ninety-six hours incubation at 37^0 C under aerobic conditions.

Haemolysis and pigment production were assessed after twenty-four and forty-eight hours growth on 5 per cent horse blood agar.

Catalase production was determined after twenty-four hours incubation on brain heart infusion agar (Difco Laboratories, Detroit, USA).

The production of urease was determined using Christensens urea agar (Merck, Darmstadt, Germany). Inoculated slopes were inspected after one, twenty-four and forty-eight hours.

Liquefaction of gelatin was assessed by adding 15 per cent mercuric chloride to 0,4 per cent gelatin agar plates (trypticase soy agar base, BBL Microbiology Systems, Cockeysville, USA) after four days incubation at 37^0 C.

Hydrolysis of esculin was determined in 0,1 per cent esculin broth (peptone water base). Broths were inspected after twenty-four and forty-eight hours incubation.

Reduction of nitrate was determined in 0,1 per cent potassium nitrate broth (peptone water base) after forty-eight hours incubation at 37^0 C.

Motility was assessed by the hanging drop method after colonies had been incubated for twenty-four hours in serum broth.

If no reaction was visible in the fermentation media after ninety-six hours, the tests were repeated after supplementation of the agar with rabbit serum. All isolates which could not be identified were then further tested using the API Coryne method (Biomerieux, Lyon, France).

Antimicrobial susceptibility testing was performed on all isolates using the Kirby-Bauer disc diffusion method. Inoculum from at least five colonies was emulsified in either peptone water or serum broth and the density standardized to that of a Mac Farland 0,5 $BaSO_4$ standard. The surfaces of three 5 per cent horse blood agar plates were swabbed with the broth suspension and antibiotic discs were applied to the agar surface within fifteen minutes, by multidisc dispensers. After twenty-four hours incubation aerobically at 37^0 C, zone sizes were measured with callipers. In the case of some of the more fastidious corynebacteria, use of a hand lens was necessary to accurately measure zone sizes. The antibiotics tested and

their respective disc potencies were as follows: vancomycin (30ug), norfloxacin (10ug), amikacin (30ug), gentamicin (10ug), tobramycin (10ug), erythromycin (15ug), cefotaxime (30ug), ceftriaxone (30ug), nitrofurantoin (300ug), penicillin (10 I.U.), oxacillin (1ug), sulphamethoxazole (300ug), ampicillin (10ug), trimethoprim (15ug), nalidixic acid (30ug) and cefazolin (30ug).

The following information was collected on every traceable study and control patient.

a. Age and sex.

b. Length of stay in hospital prior to the isolation of corynebacteria from the urine. A nosocomial infection was defined as one in which the organism was isolated after seventy-two hours of hospitalization. This is a presumed figure since urine specimens are not taken routinely on admission.

c. Signs and symptoms of urinary tract infection including dysuria, frequency of micturition, nocturia, urgency, incontinence, suprapubic and/or loin pain or tenderness, macroscopic haematuria, passage of stones and malodorous urine.

d. Underlying diseases especially urological disease, including debilitating disease of any system, malignancy, autoimmune disease and previous urinary tract infection.

e. Previous urological manipulations, including transurethral or suprapubic catheterization, cystoscopy, or surgery involving any part of the genitourinary tract.

f. Previous and current antimicrobial, cytotoxic and steroid therapy.

Between 15 June and 31 October 1991, all urine specimens received were cultured on a selective medium previously described by De Briel et al (1991), in addition to

the standard procedures described previously. The selective medium was designed to increase the yield of multiresistant, fastidious, lipophilic corynebacteria, especially Corynebacterium urealyticum and Corynebacterium jeikeium.

The selective medium of De Briel et al was made as follows: 5 per cent sheep blood agar (trypticase soy base, BBL Microbiology Systems, Cockeysville, USA), was supplemented with tween 80 (1 per cent v/v, Merck, Darmstadt, Germany), ticarcillin (100mg/l, SmithKline Beecham PLC, Brockam Park, United Kingdom), fosfomycin (50mg/l, Sigma Chemical, Poole, UK), cefotaxime (32mg/l, Roussel Laboratories, Paris, France) and 5-fluorocytosine (200mg/l, Roche Pharmaceuticals, Basel, Switzerland).

All cases in which the selective medium plate yielded $>10^4$ cfu/ml corynebacteria, were included in this second part of the study, even if another pathogen grew on the blood or Mac Conkey agar plates in greater numbers.

Corynebacteria were identified by the conventional methods described above as well as by the API Coryne method. Susceptibility testing was performed on all isolates as described above. Patients were assessed using the same criteria previously specified.

Statistical analysis

Proportions were compared using Fishers exact test on Epistat. A p value of 0,05 was regarded as significant.

4. RESULTS OF THE FIRST PART OF THE STUDY.

During the five month period in which the first part of the study was conducted, 7,912 urine specimens were cultured. Forty-four (0,56 per cent), of these specimens yielded $> 10^4$ colony forming units (cfu) corynebacteria/ml of urine. Five of these isolates were Corynebacterium urealyticum and since this organism is a known urinary pathogen, these five cases were analysed separately from the other thirty-nine cases. In nine cases, another organism was also present in the urine at less than ten cfu/ml. Forty-four "E. coli" controls, sixteen "S epidermidis" controls and forty-four "no growth" controls were selected by the method described in chapter three.

4.1 Clinical and laboratory data

a. Cases with corynebacteriuria (excluding Corynebacterium urealyticum)

i. Patient data

Twelve (30,8 per cent) samples were received from male patients and twenty-seven (69,2 per cent) from female patients. The age range of the patients extended from seventeen to seventy-eight years with a mean age of 43,6 years. In ten cases records were not traceable, or data were incomplete. Eleven (28,2 per cent) infections were presumably nosocomially acquired since the organisms were isolated after seventy-two hours of hospitalization. Eleven (36,6 per cent) of the patients complained of symptoms, or demonstrated signs which could be related to urinary tract infection. Ten patients complained of dysuria, five, of nocturia, five, of lower abdominal pain or clinically tender lower abdomen and two, of incontinence.

Underlying debilitating disease, or a condition predisposing to urinary tract infection was present in twenty-four (64,9 per cent) patients. (See table 4.2). A history of urological manipulation was present in only one patient, who had undergone a renal transplant.

Two (6 per cent) of the patients had received antimicrobial therapy prior to collection of the urine specimen. Two (5,4 per cent) patients were on immunosuppressive therapy, one receiving cyclosporin, imuran and prednisone for anti-rejection therapy and the other, prednisone for muscular dystrophy.

ii. Urine data

Thirty (77 per cent) urine specimens demonstrated pyuria and sixteen, (41 per cent) haematuria. Granular casts were present in four specimens, amorphous urates in two and calcium oxalate crystals, amorphous phosphates, leucocyte casts and waxy casts in one urine sample each.

b. Cases with Corynebacterium urealyticum infection.

Corynebacterium urealyticum was isolated in pure culture from the urine of five male patients. (Table 4.1). The patients ranged in age from twenty-two to seventy years with a mean of 54,6 years. Four reported symptoms of urinary tract infection, the fifth being comatose. Four had an underlying debilitating condition and one had recurrent urinary tract infection. One had obstructive uropathy, resulting from benign prostatomegaly. Three were catheterized, either intermittently or on a long term basis and one had a condom drain. Three were receiving antimicrobial therapy prior to and at the time the urine specimen was collected.

All the urine specimens revealed pyuria and microscopic haematuria. Three had an alkaline pH of 8,5 and two contained triple phosphate crystals. The three alkaline urine specimens produced $>10^5$ cfu/ml, whereas the other two, with pH <7, produced only $5x10^4$ and $1x10^4$ cfu/ml.

c. Escherichia coli controls

i. Patient data

Thirty (68,2 per cent) patients were female and fourteen (31,8 per cent) were male. Records were not traceable, or incomplete in four cases. The ages of the patients ranged from fifteen to eighty years, the mean age being 42,4 years. Fourteen (35 per cent) infections were presumably nosocomially acquired, the organism being isolated more than seventy-two hours after admission to hospital.

Thirty-three (82,5 per cent) patients demonstrated signs of, or complained of symptoms related to urinary tract infection. Twenty-four complained of dysuria, seventeen, of increased frequency of micturition, fourteen, of nocturia, four, of haematuria, seven, of renal angle tenderness, six, of lower abdominal pain, three, of malodorous urine and one of urgency of micturition.

Thirty (68 per cent) patients suffered from an underlying debilitating disease or condition predisposing to urinary tract infection. (See table 4.2). Eight (18 per cent) patients had received recent antibiotic therapy and three (6,8 per cent) patients were receiving prednisone for autoimmune diseases. Five (11,3 per cent) patients had undergone recent urological manipulation, three being catheterized, one having a urethrostomy and one, a condom drain.

ii. Urine data

Thirty-four (77,3 per cent) specimens demonstrated pyuria and twenty-one, (47,7 per cent) haematuria. Granular casts were present in one sample.

d. Staphylococcus epidermidis controls.

i. Patient data

Ten patients (62,5 per cent) were female and six (37,5 per cent) were male. The patients ranged from seventeen to seventy-three years in age, with a mean age of 46,6 years. Ten (62,5 per cent) of sixteen infections were presumed nosocomial in origin. Four (25 per cent) patients complained of symptoms, or demonstrated signs of urinary tract infection. Four patients complained of dysuria, two, of increased frequency of micturition, two, of nocturia, two, of lower abdominal pain and two of renal angle tenderness.

Debilitating disease or predisposing conditions were present in eight (50 per cent) of the patients. (See table 4.2). Two patients had had renal transplants followed by nephrostomies and one was catheterized. Three (18,8 per cent) patients had received recent antimicrobial therapy and the two renal transplant patients were receiving cyclosporin and prednisone.

ii. Urine data

Pyuria was present in twelve (75 per cent) samples and haematuria in six (37,5 per cent). Amorphous urate crystals were seen in two samples and amorphous phosphates in one. Granular casts were noted in one specimen.

Table 4.1 Characteristics of patients from whose urine Corynebacterium urealyticum was isolated

Age, (years) Sex	Colony count ($x10^3$/ml)	Leucocyte count/ml	Erythrocyte count /ml	pH crystals,	Signs/symptoms of urinary tract infection	Underlying disease	Urological disease or manipulation	Prior antibiotic therapy
70 M	10	250 000	6 000	5,0	dysuria, frequeacy	recurrent urinary tract infection	no	cefotaxime
60 M	>100	125 000	25 000	8,5 triple phosphate crystals	dysuria, incontinence	chronic renal failure	prostatomegaly and bilateral hydronephrosis catheterized	nil
22 M	>100	12 000	750 000	8,5	dysuria, haematuria	paraplegic	clean intermittent catheterization	nil
57 M	>100	120 000	60 000	8,5 triple phosphate crystals	dysuria, incontinence	alcoholic hepatitis in liver failure.	catheterized	ampicillin, cefotaxime, amikacin
64 M	50	40 000	55 000	6,0	not known	multiple trauma	condom drain	cephradine

c. "No growth" controls

i. Patient data

Twenty-five (56,8 per cent) patients were female and nineteen (43,2 per cent) were male. The age range was seventeen to seventy-six years with a mean of forty-five years. Data were incomplete on three patients. Seven (17 per cent) patients complained of symptoms of, or had clinical signs suggesting the presence of urinary tract infection. Seven patients complained of dysuria, four, of frequency of micturition, four, of loin pain, three, of nocturia, two, of haematuria, one of lower abdominal pain and one, of malodorous urine.

Seventeen (38,6 per cent) patients suffered an underlying debilitating disease or condition predisposing to urinary tract infection. (See table 4.2). Three patients were catheterized and one had a condom drainage system. Thirteen (29,5 per cent) had received recent antibiotic therapy. None was immunosuppressed.

ii. Urine data

Twenty-three (52,2 per cent) and nineteen (43,2 per cent) of the urine specimens demonstrated pyuria and haematuria respectively. Five specimens contained amorphous urate crystals, two contained granular casts and two, contained calcium oxalate crystals.

Table 4.2 Underlying conditions present in the study and control groups.

Underlying condition	Study cases	Escherichia coli controls	Staphylococcus epidermidis controls	No growth controls
Acute renal failure	3	3	0	5
Chronic renal failure	4	3	0	0
Malignancy of a solid organ	5	2	0	6
Myeloma	0	0	0	1
Alcoholism	5	2	0	3
Tuberculosis	3	3	1	2
Autoimmune disease	0	3	0	0
Congestive cardiac failure	1	2	0	5
Pregnancy	4	4	3	1
Prostatomegaly or ca prostate	0	2	0	0
Neurological disorders	0	2	0	3
Major trauma	0	0	0	1
Previous urinary tract infection	1	1	1	0
Diabetes mellitus	4	4	4	6

4.2 Identification of corynebacteria

Thirty-four (77,3 per cent) of the forty -four isolates were identified to the species level by the methods described above. All isolates produced catalase. Ten isolates could not be identified by all methods used. The clinical and laboratory features of the different groups are presented in Table 4.3.

a. Corynebacterium group I

Thirteen isolates were identified as Corynebacterium group I. The colonies appeared dry, grey and flat, with irregular margins. Colonial size was one millimetre after twenty-four hours. All thirteen isolates reduced nitrate and utilized glucose and maltose. Two isolates fermented starch. Confirmatory API tests were carried out on three isolates. All showed the same reactions, namely the fermentation of glucose, ribose and maltose, and the production of pyrazinamidase and alkaline phosphatase.

b. Corynebacterium minutissimum

Four isolates were identified as this species. Colonies were two millimetres in diameter after twenty-four hours and appeared dry, grey and flat with irregular margins. All isolates utilized glucose and maltose and did not reduce nitrate. One isolate could not be identified by conventional means and an API was carried out. The organism fermented glucose and maltose and demonstrated pyrazinamidase and beta-glucuronidase activity.

c. Corynebacterium striatum

Five isolates were identified as Corynebacterium striatum. Twenty-four hour colonies were one to two millimetres in diameter, yellow to beige in colour, creamy in consistency, convex and circular with entire edges. All isolates utilized glucose and sucrose and reduced nitrate.

d. Corynebacterium urealyticum

Five isolates were identified as this species. Colonies were pinpoint in size, glistening and round after twenty-four hours and were better visualized after forty-eight hours. All hydrolysed urea within thirty minutes and did not utilize any carbohydrates.

e. Corynebacterium group A5

The biochemical reactions of two isolates fell within this group. The colonies were beta-haemolytic, beige to yellow, convex and circular, one to two millimetres in diameter after twenty-four hours. The isolates were motile, reduced nitrate and esculin, and utilized glucose, sucrose, mannitol and maltose.

f. Corynebacterium kutscheri

One isolate was identified as Corynebacterium kutscheri. Colonies were beta-haemolytic, dry, flat, yellow in colour with irregular margins, and two millimetres in diameter after twenty-four hours. The organism hydrolysed urea and esculin, reduced nitrate and fermented glucose, maltose, sucrose, lactose and orthonitrophenyl beta-D-galactopyranoside.

g. Corynebacterium xerosis

One isolate was identified as this species. The colony was dry, grey, irregular, flat and one millimeter in diameter after twenty-four hours. The organism fermented glucose, maltose and sucrose, and reduced nitrate.

h. Corynebacterium group A3

One isolate fell within this group. Twenty-four hour colonies were one millimetre in diameter, dry, yellow, flat and irregular. The organism was motile, hydrolysed esculin, and fermented glucose, maltose, sucrose and xylose.

i. Corynebacterium group ANF

Two isolates were identified by API as this species after conventional tests had failed to identify them. The colonial morphology of the two isolates differed, one being 1,5 millimetres in diameter, mucoid, beige and convex and the other, tiny, grey and dry. The API revealed the identical pattern in both cases, namely pyrazinamidase and alkaline phosphatase activity and no reaction in any of the other cupules.

Table 4.3 Clinical and laboratory data on different species of corynebacteria isolated from urine specimens

	Corynebacterium group I n(%)	Corynebacterium minutissimum n(%)	Corynebacterium striatum n(%)	Corynebacterium group A5 n(%)	Corynebacterium kutscheri n(%)	Corynebacterium xerosis n(%)	Corynebacterium group A3 n(%)	Corynebacterium group ANF n(%)	Corynebacterium urealyticum n(%)
Number of isolates	13	4	5	2	1	1	1	2	5
Pyuria	10 (77)	4 (100)	4 (80)	0	1	1	1	2	5 (100)
Haematuria	5 (38,5)	1 (25)	1 (20)	0	1	0	1	1	5 (100)
Signs/ symptoms of urinary tract infection	4 (36,4)	2 (50)	3 (60)	0	not known	0	0	1	4 (100)
Underlying disease	7 (53,8)	3 (75)	5 (100)	1	1	1	1	1	4 (80)
Urological manipulation or catheterization	0	0	0	0	not known	1	0	0	4 (80)
Recent antibiotic therapy	3 (23)	0	1 (20)	0	not known	0	1	0	3 (60)
Immunosuppression	1 (7,7)	0	0	0	not known	1	0	0	0

4.3 Antimicrobial susceptibilities

The number of strains susceptible to the antimicrobials tested is tabulated in table 4.4.

Table 4.4 Antimicrobial susceptibilities of isolates of corynebacteria.

Antibiotic	Number (%) sensitive	
	Other corynebacteria	Corynebacterium pseudotuberculosis
Vancomycin	39 (100)	5 (100)
Norfloxacin	38 (97,4)	4 (80)
Amikacin	37 (95)	1 (20)
Tobramycin	34 (87)	1 (20)
Gentamicin	34 (87)	1 (20)
Cefotaxime	39 (100)	1 (20)
Ceftriaxone	39 (100)	1 (20)
Ceftazidime	10 (25,6)	1 (20)
Cefazolin	34 (87)	1 (20)
Penicillin	20 (51)	1 (20)
Ampicillin	18 (46)	1 (20)
Erythromycin	19 (48,7)	0
Nitrofurantoin	6 (15,4)	0
Trimethoprim	4 (10,3)	0
Sulphamethoxazole	3 (7,7)	0
Nalidixic acid	1 (2,6)	0

4.4 Discussion

a. Clinical and laboratory data.

The isolation rate of corynebacteria from urine specimens in this study was 0,56 per cent. This is lower than the rate of 2 per cent obtained by Maskell et al in the United Kingdom (1990). It is possible that this difference may arise when a third world hospital is compared with a first world hospital. In the latter case, more patients are likely to be immunocompromized, or subject to complicated surgical procedures and therefore, prone to infection with organisms which normally form part of commensal skin flora.

Due to the low isolation rate of Staphylococcus epidermidis in this study, it was possible to collect only sixteen controls by applying the selection procedures described in chapter three.

The 2:1 female to male ratio obtained in this study corresponds to the ratio obtained in Maskell's study (1990) and probably reflects the fact that urinary tract infections are more common in females due to anatomical considerations. This fact is corroborated by the female to male ratio in the Escherichia coli control group.

The presence of pyuria is not significantly different when the study cases are compared with the E. coli controls (table 4.5), or the Corynebacterium urealyticum group (table 4.7), whereas the difference is significant when the study cases are compared with the "no growth" controls ($p = 0,04$), (table 4.6), which would suggest a pathogenic role for these organisms. The incidence of pyuria in the corynebacteria group, is however much higher in this study (77 per cent), than in Maskell's study (44 per cent) (1990). An alternate explanation is that the growth of corynebacteria or Staphylococcus epidermidis reflects vaginal and /or skin contamination, the source of leucocytes being from the vagina rather than from the urinary tract. To support this observation, is the finding that a significantly larger number of patients

in the E. coli group had signs or symptoms of urinary tract infection than in the study group (p=0,0002). In addition the difference between the study group and the Corynebacterium urealyticum group with respect to symptomatology approaches significance (p=0,06). These data suggest that in this hospital, techniques for collecting urine specimens are poor, leading to contamination of urine and that signs and symptoms are a better indication of infection of the urinary tract than the presence of pyuria. This is supported by the lack of a significant difference between the study group and the "no growth" controls with respect to signs and symptoms of urinary tract infection.

The incidence of microscopic haematuria was significantly higher in the Corynebacterium urealyticum group than in the other corynebacterial group table 4.7). In addition , more patients in the Corynebacterium urealyticum group had been on prior antibiotic therapy. A history of prior urological manipulation, or the presence of a urinary catheter was uncommon in all the groups except for the Corynebacterium urealyticum group in which the incidence was significantly higher than in the other corynebacterial group. All these data are typical of Corynebacterium urealyticum infection and corroborate the findings of other authors (Soriano, 1990; De Briel, 1991).

Antibiotic usage prior to collection of the urine specimens was also significantly higher in the "no growth" group than in the study group, which may explain why some urine specimens with a high cell count did not yield organisms on culture.

It is interesting to note that there are no significant differences between the data obtained from the study cases and that obtained from the Staphylococcus epidermidis controls (table 4.8). Since this organism also constitutes part of normal skin flora, these findings suggest that as with Staphylococcus epidermidis isolation of corynebacteria from urine usually represents contamination but occasionally in an immunocompromized patient may indicate true infection.

Table 4.5 Comparison of study cases with Escherichia coli controls

	Study cases n(%)	Escherichia coli controls n(%)	P value
Pyuria	30/39 (77)	34/44 (77,3)	N/S
Haematuria	16/39 (41)	21/44 (47,7)	N/S
Symptoms/signs of urinary tract infection.	11/30 (36,6)	33/40 (82,5)	p=0,0002
Underlying disorder	24/37 (64,9)	30/44 (68)	N/S
Urological manipulation or catheterization	1/37 (2,7)	5/44 (11,3)	N/S
Recent antibiotic therapy	2/33 (6)	8/44 (18)	N/S
Immunosuppressive drugs	2/37 (5,4)	3/44 (6,8)	N/S

Table 4.6 Comparison of study cases with "no growth" controls

	Study cases n(%)	"No growth controls n(%)	P value
Pyuria	30/39 (77)	23/44 (52,2)	P=0,04
Haematuria	16/39 (41)	19/44 (43,2)	N/S
Symptoms/signs of urinary tract infection	11/30 (36,6)	7/41 (17)	N/S
Underlying disorder	24/37 (64,9)	17/44 (38,6)	P=0,03
Urological manipulation or catheterization	1/37 (2,7)	4/44 (9)	N/S
Recent antibiotic therapy	2/33 (6)	13/44 (29,5)	0,018
Immunosuppressive drugs	2/37 (5,4)	0/44	N/S

Table 4.7. Comparison of Corynebacterium urealyticum with the other corynebacteria.

	Corynebacterium urealyticum n(%)	Other corynebacteria n(%)	P value
Pyuria	5/5 (100)	30/39 (77)	N/S
Haematuria	5/5 (100)	16/39 (41)	p=0,04
Signs/ symptoms of urinary tract infection	4/4 (100)	11/30 36,6)	p=0,06
Underlying disease	4/5 (80)	24/37 (64,9)	N/S
Urological disorder or catheterization	4/5 (80)	1/37 (2,7)	p=0,0004
Recent antibiotic therapy	3/5 (60)	2/33 (6)	p=0,02
Immunosuppression	0/5	2/37 (5,4)	N/S

Table 4.8 Comparison of study cases with Staphylococcus epidermidis controls

	Study cases n(%)	Staphylococcus epidermidis controls n(%)	P value
Pyuria	30/39 (77)	12/16 (75)	N/S
Haematuria	16/39 (41)	6/16 (37,5)	N/S
Symptoms/signs of urinary tract infection	11/30 (36,6)	4/16 (25)	N/S
Underlying disorder	24/37 (64,9)	8/16 (50)	N/S
Urological manipulation or catheterization	1/37 (2,7)	3/16 (18,8)	N/S
Recent antibiotic therapy	2/33 (6)	3/16 (18,8)	N/S
Immunosuppressive drugs	2/37 (5,4)	2/16 (12,5)	N/S

b. Identification of corynebacteria.

Of 172 coryneforms received by the reference laboratory of the National collection of Type Cultures (NTCC) between 1965 and 1975, only 35 per cent were identified to the species level and only 43 per cent to the genus level (Coyle, 1990). These figures emphasise the difficulties encountered in the identification of corynebacteria.

The use of the API rapid coryne identification scheme (Bio Merieux, Lyons, France), has been investigated by various authors (Freney, 1991; Gavin, 1992). Gavin found that although 89,7 per cent of known pathogens such as Corynebacterium diphtheria and Arcanobacterium haemolyticum and 85,3 per cent of opportunists such as Corynebacterium jeikeium were correctly identified after twenty-four hours, the identification rate for the CDC coryneform groups was low at 36,4 per cent. Freney identified only 60 per cent of his isolates by conventional tests. Of this 60 per cent, only 65,8 per cent were identified by the API method without additional tests. Although the results of the API coryne demonstrated excellent concordance with the results of conventional methods, it appeared to show no advantage over the latter.

Examples of the difficulties encountered in the study are enumerated below:

1) The four isolates whose biochemical profile identified them as Corynebacterium minutissimum demonstrated a different morphology from that of the NTCC strain described by Jones (1986). This organism is a member of a loosely defined group of fluorescent diphtheroids and although the morphology of the strains in this study did not correspond to that of the type strain NTCC 10288, Marples (1969) described two different colonial morphologies among strains isolated from human skin, the one resembling the isolates obtained in this study.

2) Corynebacterium kutscheri is a pathogen of small rodents (Jones, 1986) and is as such unlikely to colonize man. It is therefore possible that this organism was incorrectly identified.

3) The two isolates identified as Corynebacterium group ANF differed considerably in colonial morphology and their identification is therefore also doubtful.

In no case, did the addition of lipid in the form of rabbit serum assist in the identification of the organism.

It can be seen therefore, that all isolates obtained in this study could not be identified reliably by the methods employed.

An exception to the above statement was Corynebacterium urealyticum which was easily identified by the rapid production of urease and lack of reaction in any other medium.

c. Infections due to Corynebacterium urealyticum.

Corynebacterium urealyticum was isolated from 0.06 per cent of routine urine specimens. This isolation rate is considerably lower than the 0.2 per cent obtained by Aguado et al in 1987, possibly reflecting the fact that there are fewer immunocompromized patients and patients undergoing urological manipulation in a third world setting.

All five specimens demonstrated pyuria and haematuria. The number of isolates demands caution in the comparison of these data with those of other series. In one series, the rate of pyuria and haematuria was 57 per cent and 56 per cent respectively (Soriano, 1990) and in the other study, the rates were 84 percent and 71 per cent respectively (De Briel, 1991).

The presence of triple phosphate crystals in two specimens and the alkaline pH of three specimens is typical of infection with this urealytic organism. The urine samples which did not demonstrate a high pH had low colony counts and may therefore not have represented significant infection.

Struvite crystals were present in 71 per cent of cases in one study (De Briel, 1991) and urinary pH was greater than 7.13 in 72 per cent of cases in another study (Soriano, 1987). Similarly, most case studies of Corynebacterium urealyticum infection demonstrated these phenomena in infected urine (Nadal, 1988; Sofras, 1988; van Bosterhaut, 1987; Ronci-Koenig, 1990; Morales, 1992; Ohl, 1992). It is not known whether alkaline encrusted cystitis occurred in any of the cases in this study since cystoscopic examinations were not performed.

The high incidence of underlying debilitating disease in the five patients is in keeping with the findings of other authors (Aguado, 1987; Nadal, 1988; Ronci-Koenig, 1990; Soriano, 1990; Morales, 1992; Ohl, 1992) and confirms the role of Corynebacterium urealyticum as an opportunistic pathogen.

d. Antimicrobial susceptibility testing.

One hundred per cent of the isolates in this study were susceptible to vancomycin. These results correspond to the findings of Maskell (1990). The rate of susceptibility to the quinolones, aminoglocosides and cephalosporins, with the exception of ceftazidime, was above 79 per cent. The poor susceptibility to ceftazidime is in keeping with that of other Gram-positive organisms.

More than 50 per cent of isolates were resistant to penicillin, ampicillin and erythromycin and greater than 85 per cent were resistant to the other drugs tested. These figures are higher than those obtained by Maskell (1990), which may be due to the fact that antibiotics such as penicillin, ampicillin, erythromycin and

trimethoprim/ sulphamethoxazole are used more frequently in this population. In Soweto, nursing sisters are trained to dispense certain first line antibiotics to patients with mild infections , without consulting a medical doctor.

An appropriate choice of oral antibiotic for treatment of significant urinary tract infection due to coryneobacteria, is therefore a first or second generation cephalosporin, while more serious infections should be treated with parenteral cephalosporins, aminoglycosides or vancomycin.

5. RESULTS OF THE SECOND PART OF THE STUDY

As Corynebacterium urealyticum had been identified in five patients in the first part of the study, it was decided to further evaluate the pathological relevance of the organism by using selective media. Between 15 June and 31 October 1991, 1,281 urine specimens were cultured on routine media as well as on the selective medium described in chapter 3.

5.1 Corynebacterium urealyticum

Sixteen (1,2 per cent) of the specimens yielded Corynebacterium urealyticum on the selective medium, in numbers greater than 10^4 colony forming units per millilitre (cfu/ml). In three cases Corynebacterium urealyticum was isolated from a subsequent specimen. In only two cases did the organism grow on the blood agar as well as on the selective medium and these two isolates were analysed separately from the others. In no instance did the organism grow on the Mac Conkey agar. In fifteen of the seventeen specimens, (88 per cent), another organism was isolated concomitantly with Corynebacterium urealyticum. There were four Escherichia coli isolates, three Proteus mirabilis isolates, two Enterobacter isolates, one Staphylococcus aureus isolate, three Enterococcus faecium isolates, one Enterococcus faecalis isolate and one Klebsiella isolate. In seven cases the other pathogen constituted the predominant growth.

a. Patient data.

Nine patients were female and five were male. The age range was seventeen to eighty-two years with a mean of 53,5 years. Seven (50 per cent) infections were presumably nosocomially acquired. Records were not traceable or incomplete for three of the fourteen patients. Nine (82 per cent) of eleven patients had underlying disorders which may have predisposed them to urinary tract infection. Two had

Table 1 Characteristics of patients with Corynebacterium urealyticum isolated on selective medium.

Age and sex	Leucocytes/ml urine	Erythrocytes/ml urine	pH, crystals	Symptoms	Underlying disease	Urological disease, manipulation or catheterization	Prior antibiotic therapy	Other isolates
80, female	10,000	15,000	6	unknown	unknown	unknown	unknown	Escherichia coli
79, male	15,000	none	5	unknown	cerebrovascular accident, prolonged hospitalization	catheterized	no	Enterobacter species
Second isolate*	20,000	40,000						Enterococcus faecium
?, female	250,000	none	7	unknown	unknown	unknown	unknown	Staphylococcus aureus
22, female	20,000	>750,000	6	none	post menopausal bleeding, iatrogenic uterine perforation	catheterized	yes	Enterococcus faecium
Second isolate*	10,000	>750,000	6,5					Enterococcus faecium
30, male	none	none	8, triple phosphates	unknown	head injury, comatose	catheterized	yes	Proteus mirabilis
Second isolate*	none	none	8,5					Proteus mirabilis
38, female	25,000	50,000	6,5	incontinence, frequency, urgency	none	urethrovaginal fistula	no	Escherichia coli
30, female**	30,000	none	6	dysuria	epileptic	none	yes	none

Note: Table continues overleaf.

multiple trauma, two were pregnant, one was in renal failure, one was suffering from a cerebrovascular accident, one from dementia, one had diabetes mellitus and one had carcinoma of the cervix.

Seven of nine (77,8 per cent) patients reported signs or symptoms of urinary tract infection. Five complained of lower abdominal pain or tenderness, four of dysuria, two each of frequency or urgency of micturition and one each of macroscopic haematuria, malodorous urine and incontinence. Four patients were catheterized, one had a urethrovaginal fistula and three (27 per cent) were on antibiotics before their urine was cultured.

b. Urine data

Pyuria was present in ten (71,4 per cent) specimens and haematuria in six (43 per cent). Urine pH was greater than seven in five (35,7 per cent) cases and less than seven in eleven (64,2 per cent) cases. Triple phosphate crystals were present in two specimens and uric acid crystals in one.

Table 5.1 Continued.

Age and sex	Leucocytes/ml urine	Erythrocytes/ml urine	pH, crystals	Symptoms	Underlying disease	Urological disease, manipulation or catheterization	Prior antibiotic therapy	Other isolates
43, male**	300,000	16 000	6	unknown	polytrauma	catheterized	yes	none
Second isolate*	500,000	25,000	6					none
25, male	750,000	100,000	8	lower abdominal pain, dysuria	polytrauma	catheterized	yes	Escherichia coli
?, male	none	none	6	unknown	unknown	unknown	unknown	Enterobacter species
36, female	10,000	none	8	lower abdominal pain, dysuria	pregnant	none	no	Streptococcus faecalis
80, female	15,000	none	8,5, triple phosphates	lower abdominal pain, dysuria	dementia	none	none	Proteus mirabilis
71, female	14,000	10,000	5	dysuria, malodorous urine	none	none	no	Klebsiella pneumoniae
17, female	none	none	6,5	none	pregnant	none	no	none
63, male	>750,000	45,000	6	macroscopic haematuria, dysuria	diabetes mellitus	none	no	Escherichia coli
41, female	none	none	6	lower abdominal pain, dysuria	carcinoma of the cervix	none	no	none

* In cases where Corynebacterium urealyticum was cultured from a subsequent urine specimen, the cell counts from the first specimen were considered in the analysis

** In these two cases Corynebacterium urealyticum was cultured on blood agar.

Table 5.1 Continued.

Age and sex	Leucocytes/ml urine	Erythrocytes/ml urine	pH, crystals	Symptoms	Underlying disease	Urological disease, manipulation or catheterization	Prior antibiotic therapy	Other isolates
43, male**	300,000	16,000	6	unknown	polytrauma	catheterized	yes	none
Second isolate*	500,000	25,000	6					none
25, male	750,000	100,000	8	lower abdominal pain, dysuria	polytrauma	catheterized	yes	Escherichia coli
7, male	none	none	6	unknown	unknown	unknown	unknown	Enterobacter species
36, female	10,000	none	8	lower abdominal pain, dysuria	pregnant	none	no	Enterococcus faecalis
80, female	15,000	none	8,5, triple phosphates	lower abdominal pain, dysuria	dementia	none	none	Proteus mirabilis
71, female	14,000	10,000	5	dysuria, malodorous urine	none	none	no	Klebsiella pneumoniae
17, female	none	none	6,5	none	pregnant	none	no	none
63, male	>750,000	45,000	6	macroscopic haematuria, dysuria	Diabetes mellitus	none	no	Escherichia coli
41, female	none	none	6	lower abdominal pain, dysuria	carcinoma of the cervix	none	no	none

* In cases where Corynebacterium urealyticum was cultured from a subsequent urine specimen, the cell counts from the first specimen were considered in the analysis

** In these two cases Corynebacterium urealyticum was cultured on blood agar.

5.2 Corynebacterium jeikeium.

Four (0,3 per cent) of 1,281 urine specimens yielded Corynebacterium jeikeium on the selective medium. None of these isolates grew on the blood agar or the Mac Conkey agar. In no case was Corynebacterium jeikeium isolated from a subsequent specimen. In each case another pathogen was isolated concomitantly on the blood and/or Mac Conkey agar plates. There were three isolates of Escherichia coli and one isolate of Enterobacter species. In all instances, the other pathogen constituted the predominant growth.

a. Patient data

The record of one patient was untraceable. Two patients were male and two were female. Their ages ranged from fifty-three to eighty years with a mean of sixty-nine years. Two of three patients had an underlying disorder, one suffering from carcinoma of the cervix and a urethrovaginal fistula and the other, from benign prostatic hypertrophy. Two patients complained of dysuria. No other symptoms were reported. Two patients were on prior antibiotic therapy.

b. Urine data

Pyuria was present in three (75 per cent) specimens and haematuria in one (25 per cent). All specimens had a pH of six. Calcium oxalate crystals and leucine crystals were detected in one specimen each.

5.3 Antimicrobial susceptibilities.

The antimicrobial susceptibilities of all isolates are reported in table 5.2.

Table 5.2. Antimicrobial susceptibilities of sixteen isolates of Corynebacterium urealyticum and four isolates of Corynebacterium jeikeium

Antibiotic	Corynebacterium urealyticum. Number susceptible (%).	Corynebacterium jeikeium. Number susceptible (%).
Vancomycin	16 (100)	4 (100)
Norfloxacin	15 (93,8)	1 (25)
Amikacin	9 (56,3)	1 (25)
Tobramycin	7 (43,8)	1 (25)
Gentamicin	7 (43,8)	1 (25)
Cefotaxime	1 (6,3)	1 (25)
Ceftriaxone	1 (6,3)	1 (25)
Ceftazidime	0	0
Cefazolin	0	0
Penicillin	0	0
Ampicillin	0	0
Erythromycin	2 (12,5)	0
Trimethoprim	0	0
Nalidixic acid	0	0
Nitrofurantoin	1 (6,3)	0

5.4 Discussion

Selective media have frequently been used to isolate multiresistant corynebacteria from clinical specimens (Stamm, 1979; Gill, 1981; Wichmann, 1984; Larson, 1986; Fernandez-Roblas, 1987; De Briel, 1991). The principle of using selective media to isolate these naturally slow growing corynebacteria is based on their lipophilicity and multiresistance.

The above investigators attempted to increase the isolation rate of Corynebacterium jeikeium and Corynebacterium urealyticum from sites heavily contaminated with resident flora such as skin, by incorporating into media, cephalothin, cefazolin, cefotaxime, ampicillin, ticarcillin, gentamicin, amikacin, fosfomycin, mycostatin, 5-fluorocytosine and actidione in various combinations. Similarly, tween 80, yeast extract, lecithin, histidine, sodium thiosulphate and glycerol have been incorporated into selective media to enhance the growth of these corynebacteria.

These media have increased the isolation rate of Corynebacterium jeikeium and Corynebacterium urealyticum as much as twelve-fold (Wichmann, 1984). They are however expensive and impractical because of their limited shelf life and their use has largely been confined to epidemiological studies.

The selective medium used in this study was described by De Briel et al (1991). Although in De Briel's study, the isolation rate of Corynebacterium jeikeium and Corynebacterium urealyticum increased eight fold, in eight cases the organism did not grow on the selective medium, indicating that all strains are not equally resistant and may be missed if selective media are used exclusively.

a. Corynebacterium urealyticum

Two (0,16 per cent) urine specimens yielded Corynebacterium urealyticum on routine blood agar plates during the second part of the study. A further fourteen (1,1 per cent) urine specimens yielded Corynebacterium urealyticum on the selective medium, a seven fold increase, which is similar to the increase obtained by De Briel (1991).

Non-coryneform pathogenic bacteria were cultured concomitantly on routine agar plates in 88 per cent of the cases, rendering the significance of the Corynebacterium urealyticum isolates difficult to assess. This rate is much higher than the 15,8 per cent reported by Soriano in 1990. It is well documented that Corynebacterium urealyticum can constitute part of resident skin flora, especially in hospitalized patients (Fernandez-Roblas, 1987; Soriano, 1988). Baragwanath hospital is overcrowded and in many cases, urine specimens are probably not collected according to strict aseptic procedures. The occurrence of mixed cultures from urine specimens is thus common and it is not surprising that 88 per cent of the specimens in the study yielded more than one organism. Although these patients had a high rate of pyuria, haematuria and symptoms, all these factors could be attributed to the other pathogen. Furthermore, in only five cases, was the urinary pH greater than seven and two of these specimens yielded the urealytic pathogen Proteus mirabilis. Triple phosphate crystals were demonstrated in only two specimens.

In two studies reported from Spain (Soriano, 1985; Soriano, 1990), the incidence of alkaline urine was 100 per cent and 72 per cent, and that of struvite crystaluria, 75 per cent and 55 per cent respectively. In another series (De Briel, 1991), 71 per cent of urine specimens contained struvite crystals.

Although the strains of Corynebacterium urealyticum isolated in the first part of the study were temporally separated from those isolated in the second part of the study, all the laboratory procedures were carried out by the same persons using the

same protocol. It is therefore reasonable to combine the five isolates of Corynebacterium urealyticum from the first part of the study, with the two strains isolated on blood agar in the second part of the study and compare these seven isolates with the fourteen isolates obtained on the selective medium alone (table 5). As can be seen the selective medium group yielded a significantly higher number of accompanying pathogens than the routine medium group ($p = 0.0001$) (table 5.3).

All the above data suggest that the use of selective media in situations where urine is not collected by strictly aseptic procedures may enhance the growth of contaminating skin flora and the role played by Corynebacterium urealyticum is uncertain in most cases reported in this study. Where there is significant infection by Corynebacterium urealyticum, the isolates would probably grow on the blood agar.

In four cases, Corynebacterium urealyticum was isolated twice from sequential urine specimens. In only one case, was the organism in pure culture. This patient had pyuria, haematuria, underlying disease and a history of prior antibiotic therapy and his isolate was probably significant. The other three cases all had other pathogens isolated concomitantly and only one of these had a urine pH > seven, his urine yielding Proteus mirabilis as well. These findings suggest that in hospitalised patients where groin colonization with Corynebacterium urealyticum may be encountered, sequential isolation of this organism may not always reflect infection but merely contamination due to poorly collected specimens.

Antibiotic usage in the two months prior to the isolation of Corynebacterium urealyticum was 27 per cent in the study group. This figure is far lower than the 93 percent and 90 per cent respectively, reported by European authors (Soriano, 1990; De Briel, 1991) which may possibly once again reflect the lower rate of immunocompromised patients in a third world situation.

Table 5.3. Comparison of Corynebacterium urealyticum isolates on routine media with Corynebacterium urealyticum isolates on selective media.

	Isolates on blood agar n(%)	Isolates on selective medium n(%)	P value
Pyuria	7/7 (100)	10/14 (71,4)	N/S
Haematuria	6/7 (85,7)	6/14 (43)	N/S
Signs / symptoms of urinary tract infection	5/5 (100)	7/9 (77,8)	N/S
Underlying disease	5/7 (71,4)	9/11 (81,8)	N/S
Urological manipulation or catheterization	5/7 (71,4)	5/11 (45,5)	N/S
Recent antibiotic therapy	5/7 (71,4)	3/11 (27)	N/S
Immunosuppression	0	0	N/S
Accompanying isolates	0	15/17 (88)	p=0,0001

Although the disc diffusion method for antimicrobial susceptibility testing is not recommended for slow growing organisms, by inoculating isolates into serum broth to a density equivalent to a Mc Farland 0,5 $BaSO_4$ standard and streaking it onto 5 per cent horse blood agar, zone sizes were measurable after twenty-four hours. The antimicrobial susceptibility patterns found in this study are similar to the findings of European authors. In all studies universal susceptibility to vancomycin was found (Santamaria, 1985; Fernandez-Roblas, 1987; Soriano, 1987; Fosse, 1988; Marty, 1988; Philippon, 1990; Garcia-Rodriguez, 1991). Susceptibility to norfloxacin varied between 24 per cent and 100 per cent (Santamaria, 1985; Soriano, 1987), although susceptibility to other quinolones four years later, was as low as 10 per cent (Garcia-Rodriguez, 1991). Susceptibility to aminoglycosides varied between 3,3 and 11 per cent (Santamaria, 1985; Garcia-Rodriguez, 1991). The

isolates obtained in this study were more susceptible to aminoglycosides than the European isolates. One isolate was susceptible to penicillin with an MIC of 0,06 mg/l, whereas resistance to penicillin is almost universal in European studies.

b. Corynebacterium jeikeium

As in the case of Corynebacterium urealyticum, Corynebacterium jeikeium constitutes part of commensal skin flora, with an increased incidence of colonization in hospitalized patients (Stamm, 1979; Gill, 1981; Larson, 1986; Soriano, 1988). Since the organisms are commonly isolated from the groin (Quinn, 1984; Telander, 1988), contamination of urine specimens is likely to be common. Corynebacterium jeikeium has never been implicated in urinary tract infection. In one series (De Briel, 1991), when Corynebacterium jeikeium was isolated on selective media, 80,2 per cent of cases yielded less than 10^5 organisms per millilitre. All patients were assymptomatic and all infections resolved with non specific treatment or treatment directed against accompanying pathogens.

In this study, all four strains of Corynebacterium jeikeium were cultured at less than 10^5 cfu/ml and all were accompanied by another pathogen constituting the predominant growth. Corynebacterium jeikeium could therefore not be implicated as a cause of urinary tract infection in this study.

As reported by other authors, all isolates were susceptible to vancomycin with high rates of resistance to other antibiotics (Riley, 1979; Gill, 1981; Wiehmann, 1984; Bayston, 1986; Heltberg, 1986; Larson, 1986; Colston, 1987).

6. CONCLUSIONS

In the first part of this study, the role of corynebacteria as urinary pathogens was investigated. With the exception of Corynebacterium urealyticum, corynebacteria could not be implicated as urinary tract pathogens, since the Escherichia coli control group had a significantly higher rate of symptomatology than the study group. Although the rate of pyuria was equally high in the study group and the E. coli control group, pyuria does not seem to be a reliable indicator of urinary tract infection in this hospital since even the "no growth" control group had a 52,2 per cent rate of pyuria. It seems therefore, that the presence of leucocytes in the urine of patients at Baragwanath hospital may be of very little significance, probably due to poor collection techniques. A study of this nature would probably yield better results if collection of urine specimens was strictly supervised, a virtually impossible task in this hospital.

Although the isolation rate of Corynebacterium urealyticum on routine agar was very low, being 0.06 per cent in the first part and 0.16 per cent in the second part of the study, most of the isolates appeared to be significant, the patients having high rates of pyuria, haematuria, signs and symptoms, underlying disease and prior urological manipulation. The use of selective media increased the yield of Corynebacterium urealyticum seven-fold, however most isolates were accompanied by other pathogens suggesting that the corynebacteria were contaminants. The absence of the characteristic high pH and triple phosphate crystals in most cases where Corynebacterium urealyticum was isolated on selective media further supports the opinion that the isolates were merely contaminants and not significant pathogens in most of these cases.

At a busy hospital like Baragwanath therefore, where urine specimens are not collected aseptically and Corynebacterium urealyticum is a rare pathogen, selective media should not be used routinely since it merely increases the yield of contaminating flora. Selective media could possibly be used in cases where urine

pH is high and triple phosphate crystals are demonstrated on microscopy. In addition, such urine specimens could be incubated for forty-eight hours in an attempt to increase the yield of Corynebacterium urealyticum.

Antimicrobial susceptibility testing of the isolated corynebacteria, demonstrated universal susceptibility to vancomycin and high rates of susceptibility to norfloxacin. Although most isolates of corynebacteria other than Corynebacterium urealyticum and Corynebacterium jeikeium were susceptible to the cephalosporins and aminoglycosides, the latter two species were often multiresistant. Urinary tract infection with Corynebacterium urealyticum is thus best treated with vancomycin or a quinolone.

7. REFERENCES

1. Aguado J, Ponte C, Soriano F. (1987). Bacteriuria with a multiply resistant species of corynebacterium. (Corynebacterium group D2): An unnoticed cause of urinary tract infection. J Infect Dis 156; 144-150.

2. Allen KD, Green HT.(1986). Infections due to a "Group JK" corynebacterium. J Infect 13; 41-44.

3. Arora MK, Robbins MJ, Holton J, Felmingham D. (1989). In-vitro activity of the streptogramin group of antimicrobials against Corynebacterium jeikeium and D2 coryneform bacteria. J Antimicrob Chemother 23; 458-460.

4. Athalye M, Noble WC, Mallet AI, Minnikin DE. (1984). Gas chromatography-mass spectrometry of mycolic acids as a tool in the identification of medically important coryneform bacteria. J Gen Micro 130; 513-519.

5. Athalye M, Noble WC, Minnikin D E. (1985). Analysis of cellular fatty acids by gas chromatography as a tool in the identification of medically important coryneform bacteria. J Appl Bact 58; 507-512.

6. Baker FJ, Silverton RE. (1975). Introduction to medical laboratory technology. 4th ed. Butterworths publishers.

7. Barr JG, Murphy PG. (1986). Corynebacterium striatum: An unusual organism isolated in pure culture from sputum. J Infect 13; 297-298.

8. Bayston R, Higgins J. (1986). Biochemical and cultural characteristics of "JK" coryneforms. J Clin Pathol 39; 654-660.

9. Bockwith DG, Jahre JA, Haggerty S. (1986). Isolation of Corynebacterium aquaticum from spinal fluid of an infant with meningitis. J Clin Microbiol 23; 375-376.

10. Berger S, Gorea A, Stadler J, Dan M, Zilberman M. (1984). Recurrent breast abscesses caused by Corynebacterium minutissimum. J Clin Microbiol 20; 1219-1220.

11. Bernard K, Bellefeuille M, Ewan EP. (1991). Cellular fatty acid composition as an adjunct to the identification of asporogenous, aerobic Gram-positive rods. J Clin Microbiol 29; 83-89.

12. Bolton WK, Sande M, Normansell DE, Sturgill BC Westervelt FB. (1975). Ventriculojugular shunt nephritis with Corynebacterium bovis. Successful therapy with antibiotics. Am J Med 59; 417-423.

13. Brown A. (1990). Other Corynebacteria. Principles and practice of infectious diseases, 3'rd ed; 1581-1587. Mandell GL, Douglas RG, Bennett JE, (ed). Churchill Livingstone, New York.

14. Butler W, Ahearn D, Kilburn J. (1986). High-performance liquid chromatograohy of mycolic acids as a tool in the identification of Corynebacterium, Nocardia, Rhodococcus, and Mycobacterium species. J Clin Microbiol 23; 182-185.

15. Chomarat M, Breton P, Dubost J. (1991). Osteomyelitis due to Corynebacterium group D2. Eur J Clin Microbiol Infect Dis 10; 43

16. Collins MD, Goodfellow M, Minnikin DE. (1979). Isoprenoid quinones in the classification of coryneform and related bacteria. J Gen Microbiol 110; 127-136.

17. Colt [illegible] J, Marston B, Sewell D. (1991). Necrotizing tracheitis caused by Corynebacterium pseudodiphtheriticum: Unique case and review. Rev Infect Dis 13; 73-[illegible].

18. Corina DL, Sesardic D. (1980). Profile of total mycolic acids from skin corynebacteria and from named Corynebacterium strains by gas-liquid chromatography and gas-liquid chromatography / mass spectrometry. J Gen Microbiol 116; 61-68.

19. Coyle MB, Lipsky BA. (1990). Coryneform bacteria in infectious diseases: Clinical and laboratory aspects. Clin Microbiol Rev 3; 227-246.

20. De Briel D, Langs JC, Rougeron G, Chabout P, Le Faou A. (1991). Multiresistant corynebacteria in bacteriuria: A comparative study of the role of Corynebacterium Group D2 and Corynebacterium jeikeium. J Hosp Infect 17; 35-43.

21. Digenis G, Dombros N, Devlin R, Dalla rosa S, Pierratos A. (1992). Struvite stone formation by Corynebacterium group F1: A case report. J urol 147; 169-170.

22. Ena J, Berengue J, Pelaez T, Bouza E. (1991). Endocarditis caused by Corynebacterium group D2. J Infect 22; 95-96.

23. English PC. (1985). Diphtheria and theories of infectious disease: Centennial appreciation of the critical role of Diphtheria in the history of medicine. J Pediatr 76; 1-9.

24. Fernandez-Roblas R, Prieto S, Santamaria M, Ponte C, Soriano F. (1987). Activity of nine antimicrobial agents against Corynebacterium group D2 strains isolated from clinical specimens and skin. Antimicrob Agents Chemother 31; 821-822.

25. Fosse T, Carles D, Laffont C, Lefebvre J, Bocquet J. (1988). Infections urinaires a Corynebacterium du groupe D2. Epidemie hospitaliere et sensibilite aux antibiotiques. Pathol Biol (Paris) 36; 742-745.

26. Freney J, Duperron MT, Courtier C, Hansen W, Allard F, Boeufgras JM, Monget D, Fleurette J. (1991). Evaluation of the API Coryne in comparison with conventional methods for identifying coryneform bacteria. J Clin Microbiol 29; 38-41.

27. Furness G, Evangelista A, Kaminski Z. (1977). Corynebacterium genitalium (Non specific urethritis corynebacteria). Biological reactions differentiating commensals of the urogenital tract from the pathogens responsible for urethritis. Invest Urol 15; 23-27.

28. Garcia-Rodriguez JA, Garcia Sanchez JE, Munoz Bellido JL, Nebreda Mayoral T, Garcia Sanchez E, Garcia Garcia I. (1991). In-vitro activity of 79 antimicrobial agents against Corynebacterium group D2. Antimicrob Agents Chemother 35; 2140-2143.

29. Gavin SE, Leonard RB, Briselden AM, Coyle MB. (1992). Evaluation of the rapid coryne identification system for Corynebacterium species and other coryneforms. J Clin Microbiol 30; 1692-1695.

30. Gill VJ, Manning C, Lamson M, Woltering P, Pizzo P. (1981). Antibiotic-resistant Group JK Bacteria in hospitals. J Clin Microbiol 13; 472-477.

31. Goudswaard J, van de Merwe D, van der Sluys P, Doorn H. (1988). Corynebacterium haemolyticum septicaemia in a girl with monomucleosis infectiosa. Scand J Infect Dis 20; 339-340.

32. Griffith DP. (1982). Infection-induced renal calculi. Kidney Int 21; 422-430.

33. Groseameyer P, Weisfeld A, Sonnenwirth A. (1980). Corynebacterium group JK bacterial infection in a patient with an epicardial pacemaker. Am J Clin Pathol 74; 838-842.

34. Guarderas J, Kamad A, Alvarez S, Berk S. (1986). Corynebacterium minutissimum bacteraemia in a patient with chronic myeloid leukaemia in blast crisis. Diagn Microbiol Infect Dis 5; 327-330.

35. Guarino MJ, Qazi R, Woll JE Rubins J. (1987). Septicaemia, rash and pulmonary infiltrates secondary to Corynebacterium group JK infection. Am J Med 82: 132-134.

36. Guillard F, Appelbaum PC, Sparrow F. (1980). Pyelonephritis and septicaemia due to gram-positive rods similar to Corynebacterium group E. (Aerotolerant Bifidobacterium adolescentis). Ann Intern Med 92; 635-636.

37. Hager BH, Magath TB. (1925). The etiology of incrusted cystitis with alkaline urine. J Am Med Ass 85; 1352-1355.

38. Hande KR, Witebsky F, Brown MS, Schulman CB, Anderson SE, Levine A, MacLowry JD, Chalmer BA. (1976). Sepsis with a new species of corynebacterium. Ann Intern Med 85; 423-426.

39. Harvey RL, Sunstrum JC. (1991). Rhodococcus equi infection in patients with and without Human Immunodeficiency Virus infection. Rev Infect Dis 13; 139-145.

40. Heltberg O, Friis-Moller A, Ersgaard H. (1986). Group JK diphtheroid bacteremia. Acta Path Microbiol Immunol Scand 94; 285-289.

41. Hoffmann S, Ersgaard H, Justesen T, Friis H. (1983). Fatal meningitis with group JK corynebacterium in a leucopaenic patient. Eur J Clin Microbiol 2; 213-215.

42. Hoosen AA, Resool MN, Roux L. (1991). Post traumatic ankle joint infection with Arcanobacterium haemolyticum (formerly Corynebacterium haemolyticum). S Afr J Epid Infect; 10-11.

43. Jacobs N, Perlino C. (1979). Case report "diphtheroid" pneumonia. South Med J 72; 475-476.

44. Jones D, Collins MD. (1986). Irregular, nonsporing Gram-positive rods. Bergey's manual of systematic bacteriology, vol 2, 1261-1434. Sneath PHA, Mair NS, Sharpe ME, Holt JG. (ed). The Williams and Wilkins Co.,Baltimore.

45. Krish G, Beaver W, Sarubbi F, Verghese A. (1989). Corynebacterium xerosis as a cause of vertebral osteomyelitis. J Clin Microbiol 27; 2869-2870.

46. LaRocco M, Robinson C, Robinson A. (1987). Corynebacterium pseudodiphtheriticum associated with suppurative lymphadenitis. Eur J Clin Microbiol 6; 79.

47. Larson EL, McGinley KJ, Leyden J, Cooley ME, Talbot G. (1986). Skin colonization with antibiotic-resistant (JK Group) and antibiotic-sensitive lipophilic diphtheroids in hospitalized and normal adults. J Infect Dis 153, 701-706.

48. Lipsky B, Goldberger A, Tompkins L, Plorde J. (1982). Infections caused by non-diphtheria corynebacteria. Rev Infect Dis 4; 1220-1235.

49. Lopez J, Wong F, Quesada J. (1966). Corynebacterium pseudotuberculosis. First case of human infection. Am J Clin Pathol 46; 562-567.

50. Marples RR. (1969). Diphtheroids of normal human skin. Br J Derm 81; 47-53.

51. Marshall RJ, Routh KR, MacGowan AP. (1987). Corynebacterium CDC group D2 bacteraemia. J Clin Pathol 40; 813-815.

52. Marty N, Agueda L, Lapchine L, Clave D, Henry-Ferry S, Chabanon G. (1991). Adherence and hemagglutination of Corynebacterium group D2. Eur J Clin Microbiol Infect Dis 10; 20-24.

53. Marty N, Clave D, Cancet B, Henry- Ferry S, Didier J. (1988). Corynebacterium groupe D2. Etude clinique, identification biochimique et sensibilite aux antibiotiques. Pathol Biol (Paris) 36; 460-464.

54. Maskell R, Pead L. (1990). Corynebacteria as urinary pathogens. J Infect Dis 162; 781-782.

55. Maskell R. (1989. A new look at infection of the urinary tract and its adjacent structures. J Infect 19; 207-217.

56. McNaughton R, Villanueva R, Donnelly R, Freedman J, Nawrot R. (1988). Cavitating pneumina caused by Corynebacterium group JK. J Clin Microbiol 26; 2216-2217.

57. Miller R, Brancato F, Holmes K. (1986). Corynebacterium hemolyticum as a cause of pharyngitis and scarlatiniform rash in young adults. Ann Intern Med 105; 867-872.

58. Miller R, Rompalo A, Coyle M. (1986). Corynebacterium pseudodiphtheriticum pneumonia in an immunologically intact host. Diagn Microbiol Infect Dis 4; 165-171.

59. Morales JM, Aguado JM, Diaz-Gonzalez R, Salto E, Andres A, Campo C, Praga M, Martinez MA, Leiva O, Rodriguez-Noriega A, Rodicio JL. (1992).

Alkaline-encrusted cystitis/pyelitis and urinary tract infection due to Corynebacterium urealyticum: A new severe complication after renal transplantation. Transplant Proc 24; 81-82.

60. Nadal D, Schwobel M, Von Graevenitz A. (1988). Corynebacterium group D2 and urolithiasis in a boy with megacalycosis. Infect. 16; 245-247.

61. Nathan AW, Turner DR, Aubrey C, Cameron JS, Williams DG, Ogg CS, Bewick M. (1982. Corynebacterium hofmannii infection after renal transplantation. Clin Nephrol 17; 315-318.

62. Ohl C, Tribble D. (1992). Corynebacterium group D2 infection of a complex renal cyst in a debilitated patient. Clin Infect Dis 14; 1160-1161.

63. Park Y, Hori H, Suzuki K, Osawa S, Komagata K. (1987). Phylogenetic analysis of the coryneform bacteria by 5S rRNA sequences. J Bact 169; 1801-1806.

64. Pearson TA, Hayden G, Braine HG, Rathbun HK. (1977). Corynebacterium sepsis in oncology patients. J Am Med Ass 238; 1737-1740.

65. Philippon A, Bimet F. (1990). In vitro susceptibility of Corynebacterium group D2 and Corynebacterium jeikeium to twelve antibiotics. Eur J Clin Microbiol Infect Dis 9; 892-895.

66. Pierard D, Lauwers S, Mouton M, Sennesael J, Verbeelen D. (1983). Group JK corynebacterium peritonitis in a patient undergoing continuous ambulatory peritoneal dialysis. J Clin Microbiol 18; 1011-1014.

67. Pitcher D, Soto A, Soriano F, Valero-Guillen P. (1992). Classification of coryneform bacteria associated with human urinary tract infection (Group D2) as Corynebacterium urealyticum sp. nov. Int J Syst Bact 42; 178-181.

68. Perschen R, Goodman Z, Rafsi B. (1977). Isolation of Corynebacterium species from clinical specimens. Infection and colonization. Am J Clin Pathol 68; 290-293.

69. Prescott JF. (1991). Rhodococcus equi; an animal and human pathogen. Clin Microbiol Rev 4; 20-34.

70. Quinn JP, Arnow PM, Weil D, Rosenbluth J. (1984). Outbreak of JK diphtheroid infections associated with environmental contamination. J Clin Microbiol 19; 668-671.

71. Randall A, Campbell E. (1937). Alkaline incrusted cystitis. J urol 37; 284-299.

72. Riley PS, Hollis DG, Utter GB, Weaver RE Baker C. (1979). Characterization and identification of 95 diptheroid (Group JK) cultures isolated from clinical specimens. J Clin Microbiol 9; 418-424.

73. Kolston KVI, Bodey GP. (1987). In vitro activity of various new antimicrobial agents against Group JK corynebacteria. Eur J Clin Microbiol Infect Dis 6; 491-492.

74. Roncl-Koenig T, Tan JS, File TM, Thompson RB. (1990). Infectious due to Corynebacterium group D2. Report of a case. Arch Intern Med 150; 1965-1966.

75. Ryan WJ. (1972). Throat infection and rash associated with an unusual corynebacterium. Lancet; 1345-1347.

76. Santamaria M, Ponte C, Wilhelmi I, Soriano F. (1985). Antimicrobial susceptibility of Corynebacterium group D2. Antimicrob Agents Chemother 28; 845-846.

77. Schoch P, Ferragamo M, Cunha B. (1987). Corynebacterium group D2 pyelonephritis. Urol 29; 66-67.

68. Porschen R, Goodman Z, Rafai B. (1977). Isolation of Corynebacterium xerosis from clinical specimens. Infection and colonization. Am J Clin Pathol 68; 290-293.

69. Prescott JF. (1991). Rhodococcus equi; an animal and human pathogen. Clin Microbiol Rev 4; 20-34.

70. Quinn JP, Arnow PM, Weil D, Rosenbluth J. (1984). Outbreak of JK diphtheroid infections associated with environmental contamination. J Clin Microbiol 19; 668-671.

71. Randall A, Campbell E. (1937). Alkaline incrusted cystitis. J urol 37; 284-299.

72. Riley PS, Hollis DG, Utter GB, Weaver RE Baker C. (1979). Characterization and identification of 95 diptheroid (Group JK) cultures isolated from clinical specimens. J Clin Microbiol 9; 418-424.

73. Rolston KVI, Bodey GP. (1987). In vitro activity of various new antimicrobial agents against Group JK corynebacteria. Eur J Clin Microbiol Infect Dis 6; 491-492.

74. Ronci-Koenig T, Tan JS, File TM, Thompson RB. (1990). Infections due to Corynebacterium group D2. Report of a case. Arch Intern Med 150; 1965-1966.

75. Ryan WJ. (1972). Throat infection and rash associated with an unusual corynebacterium. Lancet; 1345-1347.

76. Santamaria M, Ponte C, Wilhelmi I, Soriano F. (1985). Antimicrobial susceptibility of Corynebacterium group D2. Antimicrob Agents Chemother 28; 845-846.

77. Schoch P, Ferragamo M, Cunha B. (1987). Corynebacterium group D2 pyelonephritis. Urol 29; 66-67.

78. Selander B, Ljungh A. (1986). Corynebacterium haemolyticum as a cause of nonstreptococcal pharyngitis. J Infect Dis 154; 1041.

79. Sofras F, Yiannopoulou K, Kostakopoulos A, Dimopoulos C. (1988). Corynebacterium-induced cystitis with mucosal incrustations. J Urol 139; 810.

80. Soriano F, Aguado J, Ponte C, Fernandez-Roblas R, Rodriguez-Tudela J. (1990). Urinary tract infection caused by Corynebacterium group D2: Report of 82 cases and review. Rev Infect Dis 12; 1019-1034.

81. Soriano F, Fernandez-Roblas R. (1988a). Infections caused by antibiotic-resistant Corynebacterium group D2. Eur J Clin Microbiol Infect Dis 7; 337-341.

82. Soriano F, Fernandez-Roblaz R, Zapardiel J, Rodriguez-Tudela J, Aviles P, Romero M. (1989). Increasing incidence of Corynebacterium group D2 strains resistant to norfloxacin and ciprofloxacin. Eur J Clin Microbiol Infect Dis 8; 1117-1118.

83. Soriano F, Ponte C, Santamaria M, Aguado J, Wilhelmi I, Vela R, Cifuentes Delatte L. (1985). Corynebacterium group D2 as a cause of alkaline-encrusted cystitis: Report of four cases and characterization of the organisms. J Clin Microbiol 21; 788-792

84. Soriano F, Ponte C, Santamaria M, Castilla C, Fernandez Roblaz R. (1986). In vitro and in vivo study of stone formation by Corynebacterium group D2. (Corynebacterium urealyticum). J Clin Microbiol 23; 691-694.

85. Soriano F, Ponte C, Santamaria M, Torres A, Fernandez-Roblas R. (1987). Susceptibility of urinary isolates of Corynebacterium group D2 to fifteen antimicrobials and acetohydroxamic acid. J Antimicrob. Chemother. 20; 349-355.

86. Soriano F, Rodriguez-Tudela J, Castilla C, Aviles P. (1991). Treatment of encrusted cystitis caused by Corynebacterium group D2 with norfloxacin, ciprofloxacin, and teicoplanin in an experimental model in rats. Antimicrob. Agents Chemother 35; 2587-2590.

87. Soriano F, Rodriguez-Tudela J, Fernandez-Roblas R, Aguado JM, Santamaria M. (1988b). Skin colonization by Corynebacterium groups D2 and JK in hospitalized patients. J Clin Microbiol 26; 1878-1880.

88. Spach D, Opp D, Gabre-Kidan T. (1991). Bacteremia due to Corynebacterium jeikeium in a patient with AIDS. Rev Infect Dis 13; 342-343.

89. Stamm WE, Tompkins LS, Wagner KF, Counts GW, Thomas ED, Meyers JD. (1979). Infection due to Corynebacterium species in marrow transplant patients. Ann Intern Med 91; 167-173.

90. Stewart RG, Nowbath V, Carmichael M, Klugman KP. (1993). Corynebacterium group D2 urinary tract infection. S Afr Med J 83; 95-96.

91. Telander B, Lerner R, Palmblad J, Ringertz O. (1988). Corynebacterium group JK in a hematological ward: Infections, colonization and environmental contamination. Scand J Infect Dis 20; 55-61.

92. Tendler C, Bottone EJ. (1989). Corynebacterium aquaticum urinary tract infection in a neonate and concepts regarding the role of the organism as a neonatal pathogen. J Clin Microbiol 27; 343-345.

93. Vale JA, Scott GW. (1977). Corynebacterium bovis as a cause of human disease. Lancet; 682-684.

94. Valenstein P, Klein A, Ballow C, Green W. (1988). Corynebacterium xerosis septic arthritis. Am J Clin Pathol 89; 569-571.

95. Van Bosterhout B, Claeys G, Gigi J, Wauters G. (1987). Isolation of Corynebacterium group D2 from clinical specimens. Eur J Clin Microbiol Infect Dis 6; 418-419.

96. Van Etta L, Filice G, Ferguson R, Gerding D. (1983). Corynebacterium equi: A Review of 12 cases of human infection. Rev Infect Dis 5; 1012-1018.

97. Vanbosterhaut B, Surmont I, Vandeven J, Wauters G, Vandepitte J. (1989). Corynebacterium jeikeium (Group JK diphtheroids) endocarditis. Diagn Microbiol Infect Dis 12; 265-268.

98. Wichmann S, Wising von Koenig C, Becker- Boost E, Finger H. (1984). Isolation of Corynebacterium group JK from clinical specimens with a semiselective medium. J Clin Microbiol 19: 204-206.