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An outbreak of New Delhi metallo--lactamase-1 (NDM-1) producing Enterobacteriaceae in a South African hospital: a case-control study

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An outbreak of New Delhi metallo-β-lactamase-1 (NDM-1) producing *Enterobacteriaceae* in a South African hospital: a case-control study

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, South Africa in partial fulfilment of the requirements for the Masters in Medicine in the branch of Public Health Medicine

Johannesburg, 6 November 2014

Declaration

I, Pieter de Jager, declare that this research report is my own work. It is being submitted for the degree of Master in Medicine in the field of Public Health Medicine, at the University of Witwatersrand, Johannesburg. It has not been submitted for any other degree or examination at this or any other University.



Signed: Pieter de Jager

at Johannesburg, South Africa on this, the 6th day of November 2014.

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Abstract

Objective: New Delhi metallo-β-lactamase (NDM)-producing Gram-negative bacteria have spread globally and pose a significant public health threat. There is a need to better define risk factors and outcomes of NDM-1 clinical infection. We assessed risk factors for nosocomial infection with NDM-1-producers and associated in-hospital mortality.

Methods: A matched case-control study was conducted during a nosocomial outbreak of NDM-1-producers in South Africa. All patients from whom NDM-1-producers were identified were considered (n=105). Cases included patients admitted during the study period in whom NDM-1 producing Gram-negative bacteria were isolated from clinical specimens collected ≥48 hours after admission, and where surveillance definitions for healthcare-associated infections were met. Controls were matched for age, sex, date of hospital admission and intensive-care admission. Conditional logistic regression was used to identify risk factors for NDM-1 clinical infection and associated in-hospital mortality.

Findings: 38 cases and 68 controls were included. *Klebsiella pneumoniae* was the most common NDM-1-producer (28/38, 74%). Cases had longer mean hospital stays (44.0 vs 13.3 days; $P < 0.001$) and ICU stays (32.5 vs 8.3 days; $P < 0.001$). Adjusting for co-morbid disease, the in-hospital mortality of cases was significantly higher than controls (55.3% vs 14.7%; AOR, 11.29; $P < 0.001$). Higher Charlson co-morbidity index score (5.2 vs 4.1; AOR, 1·59; CI 95 % 1.15 – 2.18), more mechanical ventilation days (7.47 vs 0.94 days; AOR, 1.32; CI 95 % 1.10 – 1.59) and piperacillin/tazobactam exposure (11.03 vs 1.05 doses; AOR, 1.08; CI 95 % 1.02 – 1.15) were associated with NDM-1 infection on multivariate analysis. Cases had a significantly higher likelihood of in-hospital mortality when the NDM-1-producer was *Klebsiella pneumoniae* (AOR, 16.57; CI 95 % 2.12 – 129.6), or when they had a bloodstream infection (AOR, 8.84; CI 95 % 1.09 – 71.55).

Conclusion: NDM-1 infection is associated with significant in-hospital mortality. Risk factors for hospital-associated infection include the presence of co-morbid disease, mechanical ventilation and piperacillin/tazobactam exposure. Rational use of intensive care, medical devises and antibiotics are essential in reducing the transmission and emergence of NDM-1 and other drug resistance Gram-negative bacteria.

CHAPTER I: INTRODUCTION

1.1 Background

A recent World Health Organization report has shown that antimicrobial resistance has risen significantly around the globe and notes that "[a] post-antibiotic era—in which common infections and minor injuries can kill—far from being an apocalyptic fantasy, is instead a very real possibility for the 21st century".[1] Antimicrobial resistance, particularly among Gram-negative bacteria is a growing clinical problem and pose a significant public health threat.[2] Although there has been recent drug development to address multi-drug resistant Gram-negatives, it is unlikely that these treatments would become commercially available in the near future.[3] With the last entirely new class of antimicrobial drug developed almost three decades ago, it is extremely important to reduce the spread of resistance through rational infection prevention and control practices informed by an understanding of disease epidemiology.[1]

Infectious diseases are caused by viruses, fungi, parasites and bacteria. *Enterobacteriaceae* are a family of rod-shaped Gram-negative bacteria and include a range of clinically important pathogens such as *Klebsiella Pneumoniae* and *Escherichia coli*. New Delhi Metallo-β-lactamase (NDM–1) is an enzyme produced by *Enterobacteriaceae* carrying the $bla_{\text{NDM-1}}$ gene which inactivates all β-lactam and carbapenem antibiotics through hydrolysis and is classified an Ambler Class B metallo-β-lactamase.[4] It is one enzyme mediated mechanism by which *Enterobacteriaceae* inhibits the action of carbapenems.

NDM-1 was first described in 2008 in a Swedish patient returning from New Delhi, India. Both *E. coli* and *K. pneumoniae* isolates from this patient carried the novel metallo-β-lactamase gene ($bla_{\text{NDM-1}}$).[5] During the subsequent three years, NDM-1 had been reported in North America, Europe, South East Asia and Australia, with most early cases of NDM-1 diagnosed in the UK having epidemiological links with the Indian sub-continent.[6] The first NDM-1 case to be detected in South Africa occurred in an 86 year old male patient in September 2011.[7]

$bla_{\text{NDM-1}}$ is plasmid mediated and readily transferred between different members of the *Enterobacteriaceae* family and other Gram-negatives.[6] It confers resistance to three major

classes of antibiotics – the β-lactams (including carbapenems), fluoroquinolones and aminoglycosides – typically reserving susceptibility to only colistin and tigecycline.[6] However, the effectiveness of colistin and tigecycline in the treatment of NDM-1 producers has not been established. Due to cost and restriction, these drugs are also not widely available in the South African public health sector. Therefore, NDM-1 producers pose a significant clinical challenge particularly in under-resourced settings.

NDM-1 poses a major public health threat for at least three reasons. Firstly, the NDM-1 resistance mechanism is highly transferable between various *Enterobacteriaceae* family members and confers high-level antimicrobial resistance to multiple classes of commonly used antibiotics. Secondly, the rapidity with which NDM-1 has spread globally. Lastly, *Enterobacteriaceae* are ubiquitous, constitute the most common gut commensals, and are responsible for the majority of clinically important bacterial infections in humans.[6,8]

1.2 Justification

In September 2012, a private hospital group approached the National Institute of Communicable Diseases' Outbreak Unit via the National Department of Health to assist with the investigation and control of an outbreak of New Delhi metallo-β-lactamase (NDM-1) producing *Enterobacteriaceae* in three private hospitals in the greater Johannesburg area.

Subsequently an investigation into all 105 cases which had been identified through the hospitals' screening programmes were undertaken to establish possible risk factors for transmission of NDM-1 and inform recommendations for outbreak control. The outbreak investigation included a review of patient clinical and laboratory records as well as patient/relative structured telephonic interviews to establish past admissions and/or international travel history.

The initial investigation provided some insights, but due to the lack of an appropriate comparator group it was not sufficient to clearly identify and quantify risk factors for NDM-1 acquisition and its associated outcomes. A case-control study would provide stronger evidence to aid in understanding the epidemiology of NDM-1 producing *Enterobacteriaceae*. A literature search suggested this to be the largest healthcare associated outbreak of NDM-1 reported to date.[9–11] Since the majority of cases were identified from the same healthcare facility and little is known about the epidemiology of NDM-1, it presented a unique opportunity to gain a better understanding of the factors associated with NDM-1 acquisition

and, in so doing, help inform strategies to prevent or control future outbreaks of multi-drug resistant organisms in South Africa and elsewhere.

1.3 Aim and Objectives

To identify risk factors associated with the acquisition of NDM-1 producing *Enterobacteriaceae* in a South African hospital and estimate its burden in terms of morbidity and mortality.

The objectives of this study are:

I. To describe a South African hospital-associated outbreak of NDM-1 producing *Enterobacteriaceae*, in particular:
    a. Number of cases over time (epidemic curve);
    b. Description of case detection rates, average time to detection and average length of stay;
    c. Description of organisms found to be producing NDM-1; and a
    d. Description of site of NDM-1 infection;

II. To describe the characteristics of confirmed cases and controls:
    a. Average length of stay for cases and controls
    b. Average time at risk for cases and controls
    c. Co-morbidities, as measured by Mortality Probability Models III and Charlson Scores, for cases and controls
    d. Average number of antibiotic doses received for cases and controls
    e. Average number of days cases and controls were exposed to selected invasive medical devises
    f. Number of in-hospital deaths amongst cases and controls

III. To determine factors associated with infection by NDM-1 producing *Enterobacteriaceae*

CHAPTER II: LITERATURE REVIEW

2.1 Introduction

Communicable disease remains a major contributor to the global burden of disease. As argued in 2007 World Health Report, the rise of emerging and re-emerging infectious diseases and drug resistant organisms poses a challenging threat to global health.[12] This chapter provides a brief overview of relevant literature to contextualize the significance of NDM-1 producing *Enterobacteriaceae*.

2.2 Communicable Disease

Despite major advances in the treatment and prevention of infectious diseases during the 20th century, communicable diseases "...continue to plague our modern world".[13] Communicable diseases are major contributors to the global burden of disease and disproportionately affect developing countries and in particular sub-Saharan Africa,[14] where infectious diseases remain the main reason for hospitalization and death.[15]

In the broader context of globalization, emerging infectious diseases like Human Immunodeficiency Virus (1981) and more recently Severe Acute Respiratory Syndrome (2001)[16], pandemic influenza (2009) and Middle Eastern Respiratory Syndrome (2012) pose a continuous threat to Global Health.[16–18] As with newly emerging diseases, the growing problem of drug resistance undermine public health efforts in disease control and elimination. Drug resistant tuberculosis, for example, has emerged as a result of failed public health efforts to control the disease and threatens to derail global efforts in tuberculosis control.[19]

Osram classically described three epidemiological transitions namely the i) age of pestilence and famine; ii) age of receding pandemics followed by iii) age of degenerative and man-made disease.[20] Drivers of communicable disease in the third transition can broadly be understood by the following. Firstly, public health failures which result in the emergence of drug resistance. Secondly, environmental drivers such as globalization and climate change which contribute to a change in infectious disease epidemiology. Thirdly, social and demographic changes such as an aging population, urbanization and increased population density and the rise in non-communicable diseases which together change the susceptibility of populations to infectious diseases.[21] Therefore, a dynamic evolutionary relationship exists between the infectious agent, host and environment in determining the spread and transmission of emerging infectious diseases and the rise of antimicrobial resistance.[22]

2.3 Burden of antimicrobial drug resistance and healthcare associated infections

Multi-drug resistance comes with significant public health, clinical and resource implications.[23,24] Although data from developed countries are lacking there is even less data from developing countries on the burden of antimicrobial resistance.[25] A number of authors have suggested the burden of drug resistance to be greater in developing countries.[25,26] As seen with methicillin resistant *Staphylococcus Aureus*, antibiotic resistance is typically born in the intensive care setting later spreading to the general hospital environment before entering the community.[26,27] Local communities colonised with drug resistant organisms through travel transmit the resistance regionally and eventually globally.[27] Infection with drug resistant organisms are typically associated with worse patient outcomes due to a reduction in the number and effectiveness of treatment options.[28] For example, in a case control study, crude in-hospital mortality among patients with blood stream infection caused by carbapenem resistant *K. Pneumonia* was 72% versus 22% amongst matched controls without bacteraemia.[29]

The Centre for Disease Control and Prevention defines healthcare associated infections "...as a localized or systemic condition resulting from an adverse reaction to the presence of an infectious agent(s) or its toxin(s). There must be no evidence that the infection was present or incubating at the time of admission to the acute care setting."[30]

Healthcare associated infections are associated with increased mortality and length of stay and therefore result in increased financial costs.[31–38] For example, it has been estimated that the direct annual hospital costs of hospital-acquired *Clostridium Difficile* alone amounts to USD 1.1 billion in the United States[39] and in the UK healthcare associated infections cost the National Health Service approximately £1 billion or approximately 1.4% of total health spend in 2003.[6,40] It is not known what the economic cost of healthcare associated infections is in South Africa.

Healthcare associated infections, beyond constituting a significant economic burden on health systems, are associated with high rates of morbidity and mortality. It has been estimated, based on a conservative mortality rate of 15% that healthcare associated infections "...rank amongst the most important causes of death in the developing world."[26]

2.4 Antimicrobial drug resistance

"Resistance is a nameless cloud that looms over otherwise controllable infections, but lacks the powerful status of a readily identifiable disease state to spur large-scale efforts of control"[23]

2.4.1 Mechanisms of drug resistance

Antimicrobials can be classified by their major mode of action as i) interfering with cell wall synthesis; ii) inhibiting protein synthesis; iii) interfering with nucleic acid synthesis; or iv) inhibiting of metabolic pathways.[41] Resistance can be either innate or acquired. Innate resistance results from the intrinsic characteristics of the species, for example the chromosomally coded resistance genes and efflux pumps found in *Pseudomonas aeruginosa*.[42] Under the selective pressure of antimicrobial agents, initially susceptible organisms may acquire genes encoding enzymes to inactivate antibiotics; efflux pumps to expel agents; or acquire altered cell walls.[41] Thus susceptible microbial populations become resistant by natural mutation and subsequent selection.[23] The complexity of various resistance mechanisms have increased substantially in response to increased antimicrobial usage.[41]

Acquired resistance is most commonly associated with extra-chromosomal elements introduced by other bacteria.[42] These transposable genetic elements, for example plasmids, transponsons and integrons, encoding for various drug resistant mechanism and are readily transferable to various bacteria.[42]

2.4.2 Determinants of drug resistance

Two years after the commercial introduction of penicillin in the 1940's, penicillin-resistant strains of *Staphylococcus aureus* were isolated.[43] Drug resistance has always been present as a result of natural genetic mutations, but has accelerated significantly since the introduction of antibiotic use due to an escalation of selective pressures on microbe populations.[44]

Due to the high antimicrobial exposures microbes face in the hospital setting, these environments act as an ideal breading place for resistance with new resistant isolates typically first being identified from nosocomial infections.[23] In the absence of wide spread antibiotic usage the emergence of a resistant isolate may be confined to an individual as the resistant strain will be "diluted out" by susceptible commensals.[23] However, in environments of high antibiotic usage susceptible isolates would lose their competitive advantage on the

background of low-level antimicrobial exposure to their drug resistant counterparts. This results in a dangerous imbalance in commensals with drug resistant genes.[23]

Traditionally utilization of antibiotics has been much greater in developed countries. However two recent publications have shown the exponential rise in per capita utilization of antibiotics in developing countries over the last decade as these economies grow in the context of a relatively weak pharmaco-regulatory environment (Figure 1). [45,46] During the 2000 – 2010 period global per capita consumption increased by 36% with South Africa and the other BRICS countries constituting 76% of this consumption growth.[46] As can be seen in figure 1 below, per capita consumption has increased in South Africa by up to 12% between 2000 and 2010.[46]

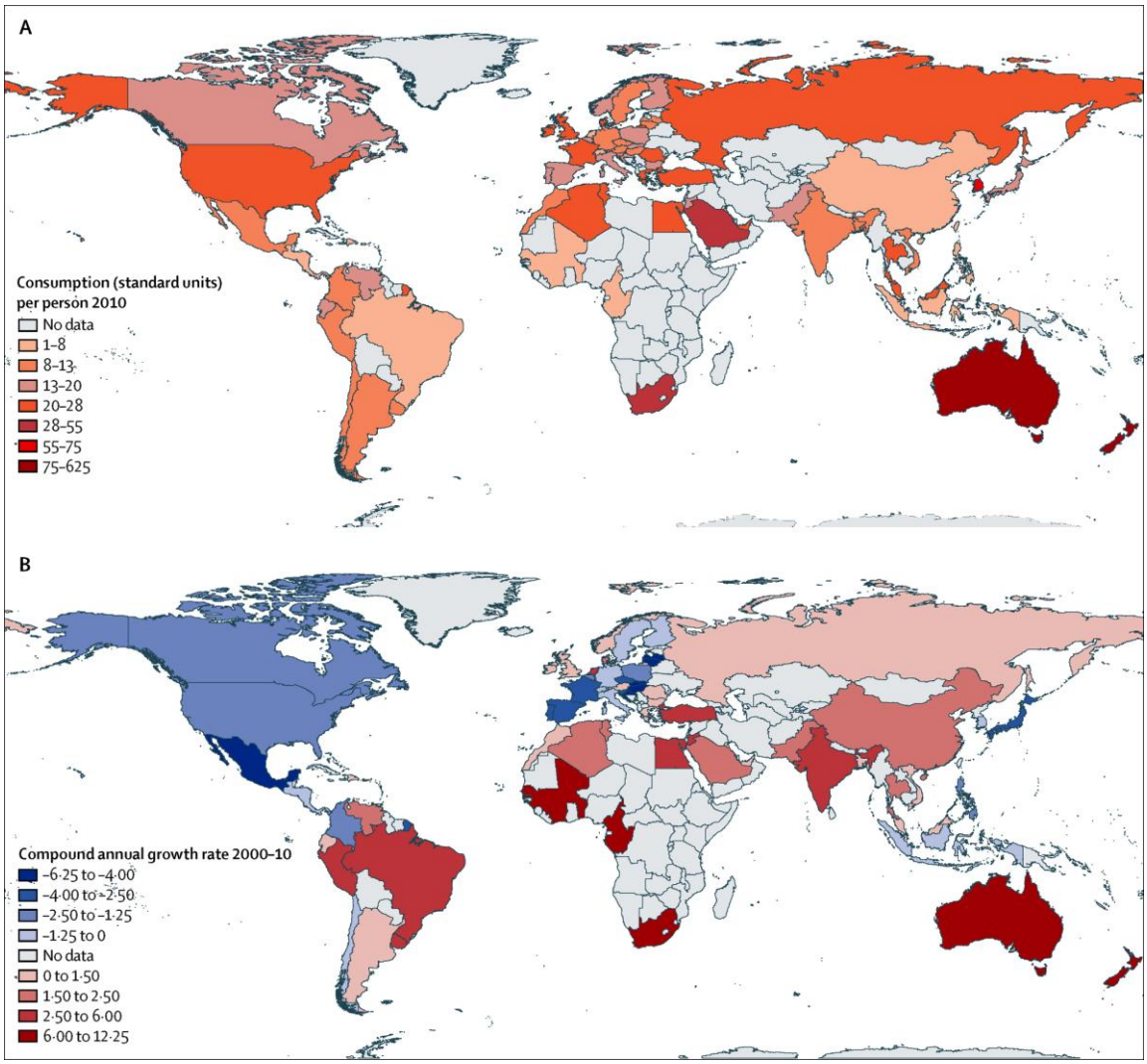


Figure 1: Antibiotic usage per capita in 2000 and compounded annual growth in antibiotic consumption 2000 – 2010
Source: Boeckel[46]

Importantly, the healthcare sector is not the only major consumer of antibiotics. Of the 22.6 million tons of antibiotics produced in the United States in 1998 only half was used by humans the remainder was consumed by the agricultural sector.[47] Chemically, antibiotics are relatively stable allowing them to persist in active form in the environment for extended periods of time.[23,47] With vast quantities of antibiotics dumped into the environment annually by the agricultural and healthcare sectors, the selection density and subsequent rise in resistance goes beyond the clinical setting and creates a deleterious ecology conducive to rising and worsening drug resistance[47] and rising rates of drug resistant community-acquired infections.[48,49] Notably, a recent study by Walsh *et al*[50] found wide spread dissemination of resistance mechanisms, including NDM-1, in environmental samples in India. These findings suggest that the transmission of resistance mechanisms between Gram-negatives are not confined *in vivo* or even the hospital setting, but occur in the environment as well.

2.4.3 Carbapenem Resistant *Enterobacteriaceae*

Resistance to multiple classes of antibiotics was first observed in *Enterobacteriaceae* in the 1950's and 1960's.[51] Resistance to β-lactams is a long recognised problem in Gram-negative bacteria and *Enterobacteriaceae*.[52] With the introduction of new classes of β-lactams, novel β-lactamases have emerged.[52,53] Carbapenem resistance, which renders organisms non-susceptible to carbapenems and as such last-line treatment, has become a growing problem over the last decade with the emergence of readily transferable plasmid mediated carbapenem-hydrolysing β-lactamases.[54,6] These carbapenemases constitute a heterogeneous and versatile group of enzymes hydrolysing β-lactams and also exhibit resistance to β-lactamase inhibitors such as piperacillin/tazobactam, making them exceedingly difficult to treat.[6,55]

Infection with Carbapenem Resistant *Enterobacteriaceae* (CRE's) has been independently associated with an increase in in-hospital mortality.[56] There is paucity in studies conducted to determine risk factors for acquiring CRE's. A study conducted in Spain with 55 cases in 2009 found mechanical ventilation, use of parental nutrition and exposure to linezolid and extended-spectrum cephalosporins to be independently associated with acquiring carbapenem-nonsusceptible *Klebsiella pneumoniae*.[57] A German study with 13 cases conducted in 2006 showed severity of underlying disease and haemodialysis to be important risk factors for acquiring carbapenem-resistant *Acinetobacter baumannii*.[58] In a Brazilian

study involving a total of 86 cases, invasive medical devices (mechanical ventilation, urinary catheterisation and central venous catheterisation), hepatic transplantation, severity of underlying illness and exposure to carbapenems and/or third generation cephalosporins were associated with increased risk of acquiring carbapenem-resistant *Acinetobacter baumannii* in an intensive care unit setting.[59]

2.4.4 New Delhi Metallo- β-lactamase 1

In 2008 a novel carbapenemase in the metallo-β-lactamase class designated New Delhi metallo-β-lactamase (NDM-1) was identified in a Swedish patient returning from India.[5] The first case of NDM-1 in South Africa was identified in September 2011.[7] $bla_{\text{NDM-1}}$ is plasmid mediated and associated with numerous other resistance determinants conferring resistance to β-lactams, fluoroquinolones and aminoglycosides resulting in significant treatment option limitations.[6,60] Sensitivity to tigecycline and polymyxins (e.g. colistin) are typically reserved although the efficacy of these treatment options have not been established and drug toxicity particularly with colistin poses further clinical challenges.[61] Compared to other carbapenemase types, NDM-1 displays a broader spectrum of antimicrobial resistance and its global spread has been singularly rapid; notably, it has been detected in diverse species and genera of Gram-negative bacteria.[62,63] NDM-1-producers have been documented on every continent except Antarctica,[64–66] with increasing reports of transmission and acquisition of NDM-1-producers both in healthcare facilities and in the community.[67,68]

In Europe, NDM-1 has been most commonly associated with *K. pneumoniae* and *E. coli* with a total of 77 cases reported across 13 countries from 2008 – 2010.[9] From these cases, increased risk for NDM-1 infection has been associated with the presence of underlying co-morbid disease, history of invasive medical procedures, and a travel history to the Indian subcontinent (India and Pakistan) or Balkan states, especially if medical treatment was received.[9] NDM-1 has been detected in a number of African countries, however risk factors for NDM-1 acquisition and mortality associated with NDM-1 is based on evidence from isolated cases or case-series only.[7,69,70] With limited treatment options available, slowing and preventing the spread of $bla_{\text{NDM-1}}$ will depend on an understanding of risk factors for its acquisition.

2.5 NDM-1 in South Africa

The National Institute for Communicable Diseases houses a national reference laboratory for antimicrobial resistance, the Antimicrobial Resistance Reference Laboratory (ARRL). Since November 2011 laboratories across the country have been encouraged to send possible carbapenem resistant isolates for molecular testing to the ARRL. However, isolates are not routinely sent currently. Surveillance data from the ARRL have been published in the monthly NICD Communiqué.[71]

Analysis of the data published in the Communiqué (June 2012 – August 2014) shows that between November 2011 and April 2013 a total of 37 NDM-1 cases had been identified nationally. These cases were from the private sector in Gauteng Province. There was a dramatic increase in the number of NDM-1 cases from May 2013 progressively affecting the public sector more than the private sector (Figure 2). Public sector cases have been reported from Gauteng, KwaZulu Natal, the Western Cape and the Free State. Private sector cases are primarily from Gauteng with an increasing trend in the number of cases reported from KwaZulu Natal over the period December 2013 to July 2014.

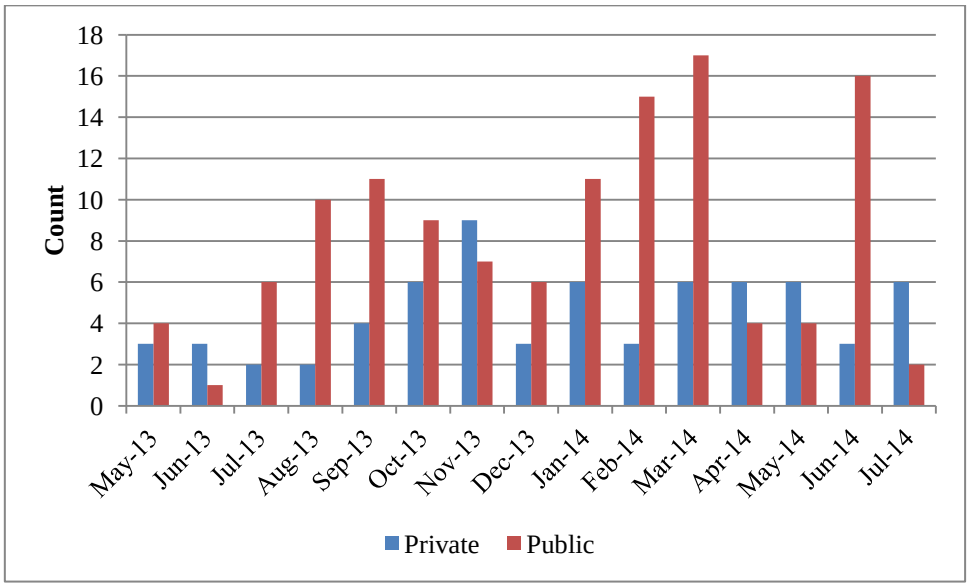


Figure 2: NDM-1 cases identified nationally by the NICD ARRL May 2013 - July 2014: Public and Private
Source: Compiled from data extracted from the NICD monthly Communiqué

As illustrated in figure 2, the trend in case detection suggests that although NDM-1 was first identified in the private sector (the first 37 cases were confined to the private sector in Gauteng, not shown in figure 2) it has become an increasingly significant problem in the public sector. Earlier identification and subsequent containment of NDM-1 could conceivably have reduced the expeditious spread of NDM-1 to the public sector and across the country.

Data from the ARRL should be treated with caution as reporting is voluntary which probably results in an underestimation of the true extent of the spread of NDM-1 in South Africa, particularly in the private sector. Further these data only provides information on place (province and sector private/public) and time, lacking any demographic or clinical information on patients from which the isolate was collected. Lastly, available surveillance data on NDM-1 in South Africa has not been analysed to identify risk factors for its acquisition or associated morbidity and mortality.

2.6 Measuring risk factors for Healthcare Associated Infections

The case-control study design is commonly used in epidemiological studies to identify risk factors for rare outcomes as well as in investigating healthcare associated infections.

In a systematic review of case-control studies investigating healthcare associated infections, Harris *et al* identify three important epidemiological considerations in designing studies, namely i) selection of the control group; ii) adjusting for time at risk; and iii) adjusting for co-morbid disease.[72]

Controls must be selected from the source population which gave rise to cases and control selection should be independent from exposures; namely, controls must be at risk of developing the outcome of interest but their selection should not be influenced by exposures of interest.[72,73] It is advised not to select, as controls, patients with a sensitive strain of the organism under consideration as this will over-estimate the effect of antibiotic exposure. Time at risk is an important confounder and must be adjusted for at either the design phase (through matching) or the analysis phase. For controls, time at risk is defined as the time from admission to discharge or death and for cases it is defined as from the time of admission to time of diagnosis.[72]

In order to account for underlying co-morbid disease, which may be causally related to the acquisition of drug resistant organisms, Harris *et al* [72] suggest matching or adjusting for it in the analysis phase.

The Charlson co-morbidity index was developed from cohort data assessing mortality rates for various co-morbid conditions.[74] Depending on the expected mortality rate for the co-morbidity present in the patient, points are assigned from which a score is calculated. The score can then be converted into 10-year predicted mortality and as such allows for a

composite measure of co-morbidity. The Charlson score has been validated as a predictor for mortality, but has not formally been validated for its ability to adjust for confounding due to co-morbid disease.[72] However, the Charlson score has been widely used to account for co-morbid illness in studies investigating healthcare associated infections,[75–77] and therefore is utilized in this study to account for co-morbid disease.

Mortality Probability Models (MPM) were developed using multi-centre cohort data of patients admitted to intensive care units and aims to predict mortality at 24 hours after admission.[78] MPM score calculation is based on fifteen clinical parameters taken on admission into an intensive care unit and is expressed as a probability, thus providing a composite score of a patient's acute presentation.[78] MPM scores only apply to ICU patients and have not been validated for persons under the age of 18 years, with acute myocardial infarction, cardiac surgery patients or patients with burns.[78] A core utility of the MPM score is for research and it has been used to adjust for acute presentation in a number of studies investigating risk factors for and mortality associated with nosocomial infections.[79,80]

Survival probabilities calculated from the MPM and Charlson scores therefore provide a measure of acute presentation and underlying co-morbidities respectively. The methods section of this report will further expand on the calculation of both these measures.

CHAPTER III: METHODS

3.1 Study design

A matched case-control study was conducted following an outbreak investigation.

3.2 Setting

The outbreak consisting of a total of 105 cases of NDM-1 producing *Enterobacteriaceae* occurred during a 17 month period (1 June 2011 to 31 October 2012) across three private hospitals in South Africa with strong referral links amongst them. This study was confined to the hospital where the majority of cases (90/105, 86%) were detected. The hospital has a total of 322 beds of which 37 beds are intensive care beds. It offers specialist tertiary-level care, acting as a referral hospital for surrounding private hospitals belonging to the same company.

In early August 2011 *Klebsiella pneumoniae* isolated from an 86-year-old male admitted following a hip fracture was found to harbour $bla_{\text{NDM-1}}$. In response to this, the first case of NDM-1 both in the hospital and the country, a rectal screening programme was instituted to identify patients colonised with NDM-1-producers, with screening criteria revisions throughout the course of the outbreak. The method of screening employed by all diagnostic laboratories throughout the outbreak was direct real-time polymerase chain reaction (RT-PCR) testing for $bla_{\text{NDM-1}}$ on dry rectal swabs. Clinical isolates demonstrating phenotypic resistance to carbapenems were also tested for $bla_{\text{NDM-1}}$ using RT-PCR. All microbiological testing was conducted in routine private diagnostic laboratories servicing the private healthcare sector.

All cases identified through the hospital screening programme during the initial outbreak investigation were reviewed and classified as suspected or confirmed cases as per the definitions in table 1.

Table 1: Case definition utilized during the initial outbreak investigation

Term	Definition
Suspected case	Isolation of any *Enterobacteriaceae* – family genus or species from a screening or clinical specimen showing resistance to carbapenems as determined by the following antimicrobial susceptibility testing (AST) methods: disk diffusion, MIC, or E-test.
Confirmed case	Presence of NDM-1 resistance gene in a screening specimen/clinical specimen/isolate as determined by RT-PCR methods.

3.3 Study population and sampling

The study population included all patients admitted to the hospital during the study period 1 June 2011 to 31 October 2012.

All 90 cases identified at the hospital through the screening programme were included in the description of the outbreak (Objective 1) with a subsequent matched case control study involving a subset of the 90 cases employed to identify risk factors for the acquisition of NDM-1 infection and associated in-hospital mortality (Objective 2 – 5).

3.3.1 Selection of cases and controls

For the case-control study the case definition that had been used during the outbreak investigation was refined as reflected in table 2 below. Only confirmed cases, as per table 2, were eligible for inclusion in the case-control study. Cases were defined as patients in whom *bla*$_{\text{NDM-1}}$ was detected on an isolate from a specimen collected at least 48 hours after admission and the infection was categorised as a healthcare-associated infection as per the Centers for Disease Control and Prevention/National Healthcare Safety Network definitions.[81] Potential cases were excluded if *bla*$_{\text{NDM-1}}$ was detected on rectal screening alone, or where clinical records were incomplete.

Table 2: Case definition utilized in the case-control study

Term	Definition
Suspected case	isolation of any *Enterobacteriaceae* – family genus or species from a clinical specimen showing resistance to carbapenems as determined by the following antimicrobial susceptibility testing (AST) methods: disk diffusion, MIC, or E-test. Isolate must have been identified at least 48 hours after admission and cause invasive disease (CDC guidelines) *Pseudomonas* and *Acinetobacter* - excluded
Confirmed case	presence of NDM-1 resistance gene in a clinical isolate collected at least 48 hours after admission as determined by PCR methods and classified as causing invasive disease as per CDC guidelines

As shown in figure 3 below, after exclusion of cases not fulfilling the inclusion criteria, 40 cases remained and three controls were matched to each case for:

i. sex (male/female);
ii. age (+/- 5 years);
iii. date of hospital admission (+/- 14 days); and

iv. Intensive care unit admission (yes/no).

Where more than three eligible controls were identified on the hospital's electronic database, three controls were randomly selected. Controls were excluded if they had *bla*$_{NDM-1}$ detected on any sample during the hospitalisation period, if patient records were incomplete or missing, or if the patient was admitted for less than 48 hours.

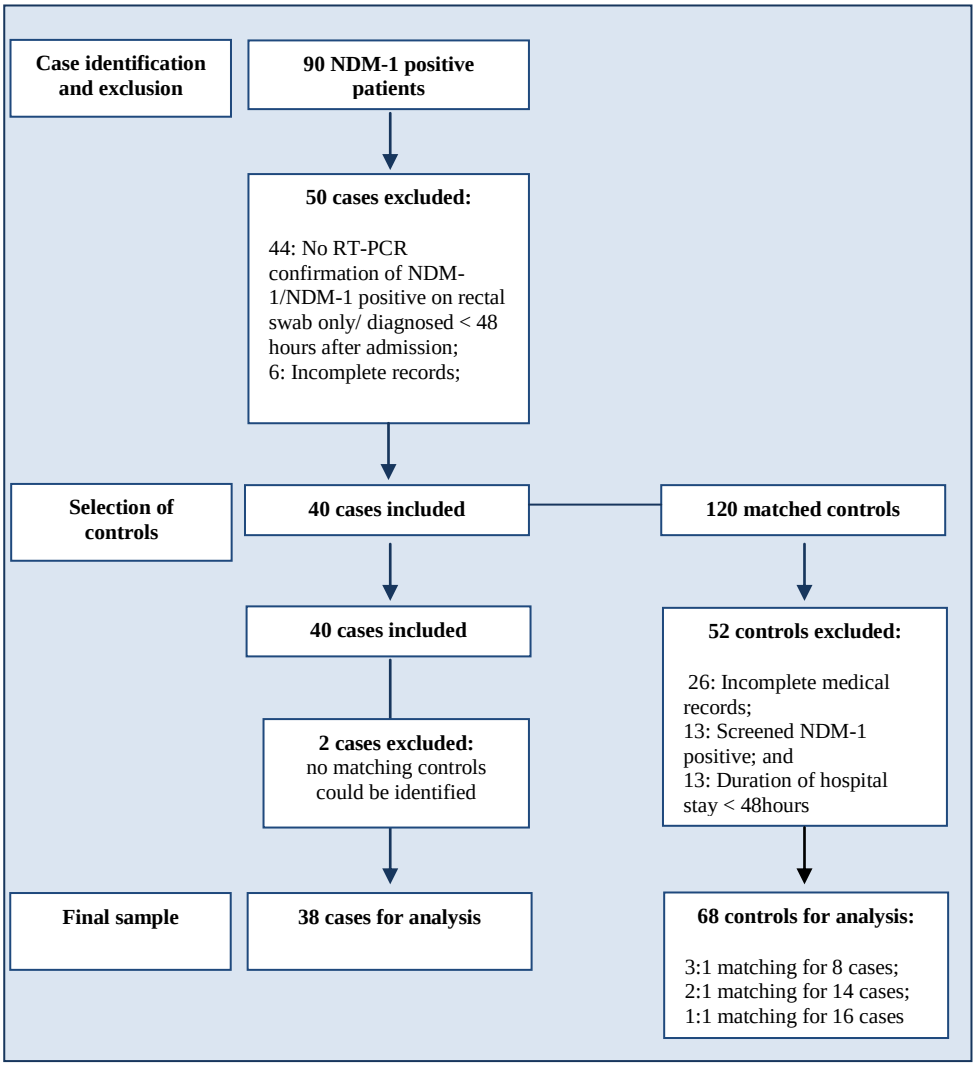


Figure 3: Outline of study design and selection of cases and controls

No controls could be found meeting the matching criteria for two cases and both these cases were therefore excluded from the analysis. For three cases only two matching controls could be identified. Another 52 controls were excluded for missing/incomplete medical records (n=26), record of screening NDM-1 positive on dry rectal swab (n=13) or being admitted for less than 48 hours (n=13). The final sample for the case-control study consisted of 38 cases and 68 controls.

3.4 Measurement

3.4.1 Data Collection

In order to describe the initial outbreak data on all 90 potential cases were collected during August – November 2012. Variables collected to describe the outbreak include date of hospital and ICU admission, date of NDM-1 diagnosis, date of discharge from hospital or ICU, date of (in-hospital) death, site of NDM-1 infection and NDM-1 producing isolates (speciation). The total number of general and ICU admission per week from 1 June 2011 to 31 October 2012 were also collected to calculate attack rates for the hospital overall and the ICU in particular. Data on the following variables specifically required for matching were also collected: ICU admission, sex and age.

Description of cases and controls involved the collection of travel, previous hospitalization and clinical data. All the above variables were also collected for controls. Additional independent variables collected for both cases and controls were inputs for the calculation of MPM III and Charlson co-morbidity index scores (see table 3 and 4 below); past travel history; history of past hospitalization or chronic care; number of doses of a carbapenem, aminoglycoside, 3^{rd} /4^{th} generation cephalosporin, fluoroquinolone, piperacillin/tazobactam and corticosteroids received; number of days of mechanical ventilation, urinary catheterization; central venous line; haemodialysis and parenteral nutrition received. Surgical history including history of receiving an endoscopic procedure and extracorporeal membrane oxygenation were also collected. All in-hospital deaths were recorded.

Data for both cases and controls were collected from four sources:

I. ***Clinical records*** were reviewed and data were extracted using a data collection tool
II. ***Billing records*** were obtained from the hospital and compared to the data obtained from the clinical record review.
III. ***Laboratory investigation results*** were obtained from private laboratories servicing the hospital.
IV. ***Structured telephonic interviews*** were conducted to determine previous travel history, previous hospital or chronic care admission.

3.4.2 Data variables

Table 5 below provides a summary of the variables collected and the data sources.

3.4.2.1 Dependent variables:

The two primary outcome variables of interest were i) NDM-1 infection as defined in section 3.3.1 above; and ii) death which was defined as any case of death in-hospital before discharge.

3.4.2.2 Independent variables:

Exposure data for cases were collected from the date of admission until the date of collection of the first sample yielding an NDM-1-producing isolate (time at risk). For controls, exposure data were collected from the date of admission until the date of discharge or death (time at risk).

Previous travel and previous hospital/chronic care admission were collected through telephonic interviews and refer to the 12 months leading up to the index admission.

Length of stay refers to the total length of hospital stay and was captured in days. Length of ICU stay was also captured in days and refers to the total duration of ICU stay. Data on select medical devices and procedures were collected based on previously reported risk factors for healthcare associated infection in the literature. These include the number of days a patients had a central venous line or a urinary catheter in situ; the number of days a patient was mechanically ventilated; received parenteral nutrition or haemodialysis. Receipt of extracorporeal membrane oxygenation (ECMO) was recorded as yes or no.

Antibiotics received during admission were recorded as number of doses received. All carbapenems (ertapenem, imipenem, doripenem); aminoglycosides (amikacin, gentamycin, tobramycin); fluoroquinolones (levofloxacin, ciprofloxacin, moxifloxacin); third and fourth generation cephalosporins (cefepime, ceftriaxone) and piperacillin/tazobactam doses were recorded.

Surgical records were also reviewed and categorized as abdominal/thoracic surgery versus other (mainly orthopedic)/no surgery. Similarly, patients who had undergone endoscopic procedures were recorded as endoscopy yes versus no endoscopy. Patients' HIV status was captured from clinical or laboratory records and recorded as a binary variable: HIV positive or HIV negative.

3.4.2.2.1 Components of Charlson co-morbidity index

The Charlson co-morbidity index, from which 10 year survival probabilities can be calculated, require information on 16 co-morbid conditions. Each co-morbidity (and depending on the severity, e.g. diabetes with and without end organ damage) a score is given as per table 3 below. These scores are then added up to give an age-unadjusted Charlson co-morbidity index. The index can be adjusted for age by adding additional points depending on the patient's age.

Table 3: Co-morbidity components and scoring of Charlson co-morbidity index

Score	Co-morbidity component
1	Myocardial Infarction (history only, no ECG changes required)
	Congestive cardiac failure
	Peripheral vascular disease (including aortic aneurysm of > 6 cm)
	Cerebrovascular disease
	Dementia
	Chronic pulmonary disease
	Connective tissue disease
	Peptic ulcer disease
	Mild liver disease (includes chronic hepatitis, no portal hypertension present)
	Diabetes without end organ damage (exclude if controlled on diet alone)
2	Hemiplegia
	Moderate or severe renal disease
	Diabetes with end organ damage (Nephropathy, neuropathy or retinopathy)
	Any non-metastatic solid tumour (exclude if tumour free for > 5 years)
	Acute or chronic Leukaemia
	Malignant lymphoma
3	Moderate or severe liver disease (signs of portal hypertension)
6	Metastatic solid tumour
	AIDS (not just HIV positive, WHO criteria)
	Adjusting for Age
0	< 40 years of age
1	41 – 50 years
2	51 – 60 years
3	61 – 70 years
4	> 70 years of age

3.4.2.2.2 Components of MPM score

Table 4 provides the 16 components required to calculate MPM-III scores. Based on whether a clinical or physiological component was present at the time of ICU admission or not a probability of in-hospital mortality is calculated (MPM score). The MPM score is calculated utilizing weighted beta-coefficients. These coefficients were estimated from the ICU and mortality data of some 125 000 ICU patients across 135 ICU's.[82]

Table 4: Components required for the calculation of MPM scores

Components	Present Yes/No
Medical or unscheduled surgical admission	
CPR prior to admission	
Coma (GCS 3-5) [1]	
Tachycardia (HR >150 bpm)	
Hypotension (SBP <90 mmHg)	
Mechanical ventilation within 60 minutes of admission	
Acute renal failure [2]	
Cardiac dysrhythmias	
Cerebrovascular accident	
Intracranial mass effect	
Gastrointestinal bleeding	
Metastatic carcinoma [3]	
Cirrhosis	
Chronic renal insufficiency [4]	
Zero factors [5]	
Full code status [6]	

[1]doesn't include patients whose coma due to overdose or neuromuscular blockade; [2]doesn't include pre-renal azotemia; [3]distant metastasis only, doesn't include lymph node involvement; [4]long-standing creatinine > 177 µmol/L; [5]elective surgical patients with no other MPM risk factors other than age; [6]decision taken to resuscitate if necessary

Table 5 below provides a summary of all the variables collected for this study, including data sources, definitions and key considerations in data management.

Table 5: Description of variables

Variable	Definition	Management	Source	Characteristics
Number of potential cases	Either a suspected or a confirmed case as per definitions in table 1	Described as cases per week	Review of clinical records	Count
Number of cases	Confirmed cases as per definition in table 2	Utilized in case control study	Review of clinical records	Count
Number of general hospital admissions	All patients admitted to the hospital between 1 June 2011 to 31 October 2012	Calculation of attack rates	Hospital electronic admission database	Count
Number of ICU admissions	All patients admitted to the hospital's ICU between 1 June 2011 to 31 October 2012	Calculation of attack rates	Hospital electronic admission database	Count
Date of hospital admission	Date on which patient was first admitted as an inpatient		Clinical Records	Date
Date of ICU admission	Date on which patient was first admitted to ICU		Clinical Records	Date
Date of NDM-1 diagnosis	Date on which NDM-1 producing isolate was collected from the patient		Clinical Records	Date
Date of discharge	Date on which patient left the hospital		Clinical Records	Date
Date of death	Date on which patient passed away (if applicable), refers to in-hospital deaths only.		Clinical Records	Date
Length of hospital stay	Total duration of hospital stay	Calculated from date of admission to death/discharge expressed in days	Clinical Records	Continuous (days)
Length of ICU stay	Total duration of ICU stay	Calculated from date of ICU admission to death/discharge in/from ICU expressed in days	Clinical Records	Continuous (days)
Time at risk	Cases: date of admission until the date of NDM-1 diagnosis Controls: the date of admission until the date of discharge or death	Calculated from date of admission to date of NDM-1 diagnosis or date of discharge/death	Clinical Records	Continuous (days)
Time to NDM-1 detection	Duration in days from time of admission to date of NDM-1 diagnosis	Calculated from date of hospital admission to date of NDM-1 diagnosis	Clinical Records	Continuous (days)
Number of deaths	All patients included in the study whom died in-hospital, namely before being discharged	Stratified by presence of NDM-1 infection, namely cases and controls	Clinical Records	
NDM-1 producing isolates	Speciation of isolate found to be harbouring $bla_{\text{NDM-1}}$		Clinical Records	Nominal

Table 5 continue				
Site of NDM-1 infection	Based on review of clinical records and CDC criteria,[81] the site of primary infection		Clinical Records	Nominal
Travel	History in the 12 months leading up to the date of hospital admission of travel outside the borders of the Republic of South Africa		Telephonic Interview	Binary
Previous hospitalization/ Chronic care	Admission to any hospital or chronic care facility in the 12 months leading up to the date of hospital admission		Telephonic Interview	Binary
Central venous line	Number of days an intravenous cannula, placed into the femoral, internal jugular or subclavian vein was in-situ	Cumulative number of days	Clinical records	Continuous (days)
Urinary catheter	Number of days an indwelling urinary catheter was in-situ	Cumulative number of days	Clinical records	Continuous (days)
Mechanical ventilation	Number of days of patient required intubation and mechanical ventilation. Excludes, for example continuous positive airway pressure (CPAP) without concomitant intubation	Cumulative number of days	Clinical records/Billing data	Continuous (days)
Extra-corporeal membrane oxygenation (ECMO)	Receipt of any ECMO		Clinical records/Billing data	Binary
Parenteral nutrition	Number of days of partial or total parenteral nutrition	Cumulative number of days	Clinical records/Billing data	Continuous (days)
Haemodialysis	Number of days a patient received haemodialysis	Cumulative number of days	Clinical records/Billing data	Continuous (days)
Carbapenem	Number of doses of ertapenem, meropenem and doripenem received	Cumulative number of doses	Clinical records/Billing data	Continuous (doses)
Aminoglycosides	Number of doses of amikacin, gentamycin and tobramycin received	Cumulative number of doses	Clinical records/Billing data	Continuous (doses)
Fluoroquinolone	Number of doses of levofloxacin, ciprofloxacin and moxifloxacin received	Cumulative number of doses	Clinical records/Billing data	Continuous (doses)
Piperacillin/tazobactam	Number of doses of piperacillin/tazobactam received	Cumulative number of doses	Clinical records/Billing data	Continuous (doses)
3/4th generation Cephalosporin	Number of doses of cefepime and ceftriaxone received	Cumulative number of doses	Clinical records/Billing data	Continuous (doses)
Steroids	Number of doses of corticosteroids received	Cumulative number of doses	Clinical records/Billing data	Continuous (doses)

Table 5 *continue*			
Surgery	Having undergone abdominal/thoracic surgery versus other or no surgery	Clinical records/Billing data	Binary
Endoscopy	Having undergone bronchoscopy, gastroscopy, cystoscopy or colonoscopy	Clinical records/Billing data	Binary
HIV status	Any clinical or laboratory documentation indicating that the patient is HIV +, if none or unknown patient assumed HIV -	Clinical records/Billing data	Binary
MPM III score	As per table 4	Clinical records	Continuous (probability)
Charlson score	As per table 3	Clinical records	Continuous
Dead	Died in hospital, namely refers to in- hospital mortality	Clinical records	Binary

3.5 Data analysis

3.5.1 Data Entry and Cleaning

Data were entered into Epi-Info version 7 and exported to Microsoft Office Excel 2007 where it was inspected for errors before being imported to STATA Version 12[83] for statistical analysis. Data were anonymised and original case investigation forms along with unique identifiers and supporting documentation (e.g. copies of laboratory reports) were filed in a locked filing cabinet at the National Institute of Communicable Diseases. Only the principle and co-investigators has access to these data.

3.5.2 Data Analysis

3.5.2.1 Description of the outbreak

Data on all the potential cases (n=90) were utilised to illustrate NDM-1 attack rate trends per 100 admissions (general and ICU) during the study period. For attack rates per 100 general admissions all potential cases (n=90) detected during each week were divided by the hospital admissions during that same week. Similarly, for attack rates per 100 ICU admissions all cases detected in ICU patients (n=83) during each week were divided by the number of ICU admission in that week. Further, data on the potential cases were used to draw a Gantt chart and epidemic curve.

Average time to diagnosis of NDM-1 was calculated utilizing the variables date of hospital admission and date of NDM-1 diagnosis. Similarly, average length of hospital and ICU stays

were also calculated using date of hospital or ICU admission and date of hospital or ICU discharge respectively. Around these point estimates, 95% CI were calculated.

Site of infection and NDM-1 producing isolates are described graphically through pie and or bar charts.

3.5.2.2 Description of cases and controls

Continuous variables such as length of hospital stay, MPM-III and Charlson scores, are described through the reporting of means and standard deviations. Two sided t-test for two groups (cases and controls) was used to compare means of continuous variables with normal distributions. Where data were not normally distributed Mann-Whitney U test was used. For differences in proportions such as previous hospitalisation or travel history, Mantel–Haenszel Chi square test was used.

3.5.2.3 Factors associated with NDM-1 infection and in-hospital mortality

Utilizing the data on the 38 cases and 68 matched controls, risk factors associated with case status were evaluated and in-hospital mortality between cases and controls were compared. Except for MPM-III scores, where its calculation would have been invalid, there were no missing clinical data in the final sample used for analysis. Where past admission, travel history or MPM-III scores were missing, observations were excluded from the analysis.

Bivariate conditional logistic regression analysis was undertaken to calculate crude odds ratio's for exposure to medical devises and interventions, antibiotics and duration of stay. Stepwise conditional logistic regression was conducted to identify predictors for case status. All exposure variables with a $P < 0.20$ at the univariate level were considered in the final multiple regression model. Significance was taken at a level of 0.05. Conditional logistic regression was further undertaken to calculate the odds of in-hospital mortality for cases and controls as well as for different sites of infection and clinical isolates. Adjusted odds ratios were calculated using multivariable conditional logistic regression.

3.6 Ethical Considerations

Verbal informed consent was obtained from all patients or their next of kin prior to conducting telephonic interviews which collected information on past hospitalization/chronic care admission and travel history. Verbal consent was obtained as this was a retrospective study and patients had subsequently relocated to various parts of the country. Consent was

captured on a consent form by the researchers. Consent to review clinical records were obtained from the hospital and all patient data were anonymized and de-linked from unique identifiers prior to analysis. Ethics approval for this study, including the consent procedure, was obtained from the Human Research Ethics Committee (Medical) at the University of the Witwatersrand, Johannesburg. (M130248)

CHAPTER IV: RESULTS

4.1 Description of the outbreak:

4.1.1 Detection of NDM-1

During the study period there were a total of 5 522 intensive care admissions and 31 644 general admissions, with an average of ±1 500 general admissions per month.

Figures 4 and 5 below illustrate the trend in NDM-1 detection during the study period for general and ICU admission respectively. Of the 105 cases 86 (82%) occurred at the hospital which was the site of this study. Of these 86 cases 83 (96.5%) required ICU admission at some point during their stay. The average detection rate between July 2011 and October 2012 was 0.39 (95% CI 0.30 – 0.48) per 100 general admissions. There were four peaks in the detection rate per 100 general admissions in March (week 31), July (week 49); September (week 58) and October (week 64) 2012.

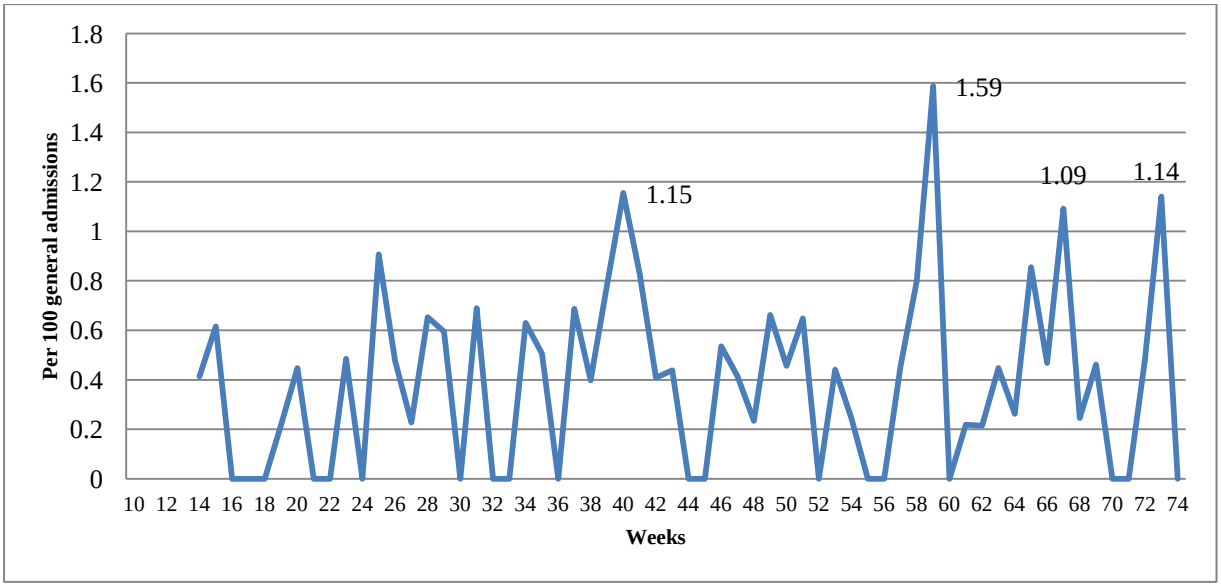


Figure 4: NDM-1detection: General admissions July 2011 to October 2012

With the majority of cases detected in ICU (96.5%) the detection rate was high at 4.65 (95% CI 3.48 – 5.83) cases of NDM-1 per 100 ICU admission with four peaks in November 2011 (week 16), March (week 30), July (week 49) and September (week 59) 2012. In September 2012 the outbreak reached a peak in terms of NDM-1 detection with approximately 1 in 5 ICU patients testing positive for NDM-1.

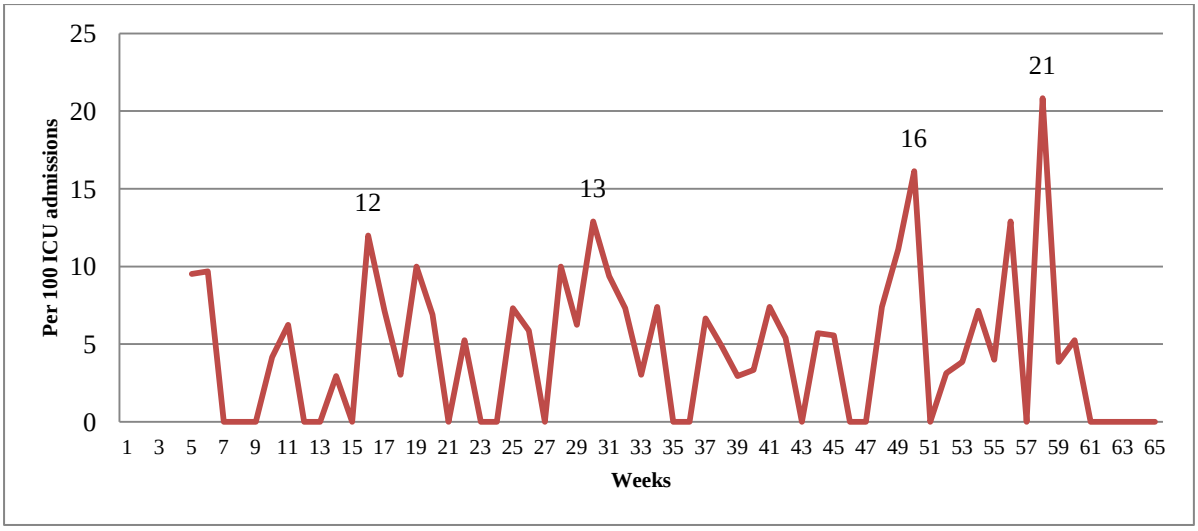


Figure 5: NDM-1 detection: ICU admissions July 2011 to October 2012

4.1.2 Epidemic curve

Figure 6 shows the epidemic curve of all the 86 potential cases identified between July 2011 and October 2012. The epidemic-curve suggests five distinct clusters, marked in figure 6 A through E.

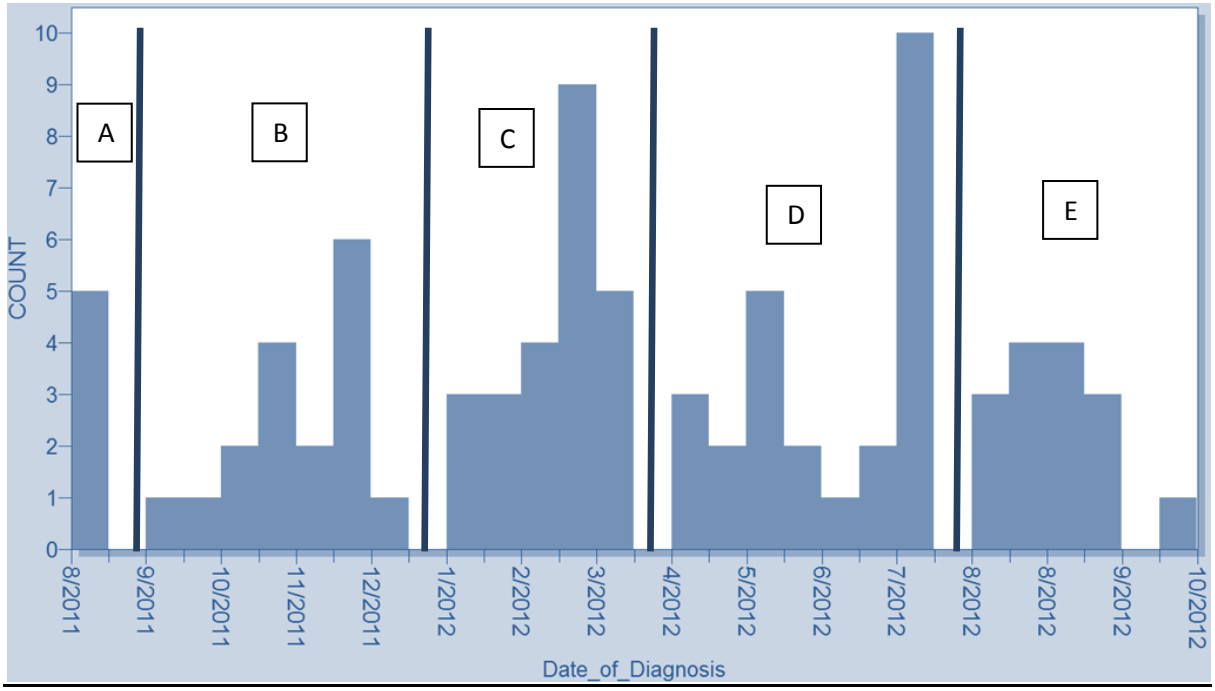


Figure 6: Epidemic curve of 86 potential NDM-1 cases

Table 6 provides a summary of the five clusters. At 25 cluster D, a 16 week period between 15 March and 14 July 2012, had the highest number of NDM-1 cases. At 2 cases per week cluster C had the highest average number of cases detected per week compared to an average of 1.39 cases per week over the entire study period.

Table 6: Number of cases detected by cluster 1 July 2011 to 31 October 2012

Cluster	Count	Time period (dd/mm/yy)	Number of Weeks	Average number of cases per week
A	5	01/7/11 – 14/08/11	6	0.83
B	17	15/08/11 – 14/12/11	16	1.06
C	24	15/12/11 – 14/03/12	12	2.00
D	25	15/03/12 – 14/07/12	16	1.56
E	15	15/07/12 – 31/10/12	12	1.25
Total	**86**	**01/07/11 – 31/10/12**	**62**	**1.39**

Figure 7 illustrates the date of admission to date of discharge (blue line) and the date of first NDM-1 detection (red dot) for all potential cases. There is clear temporal overlap between the cases.

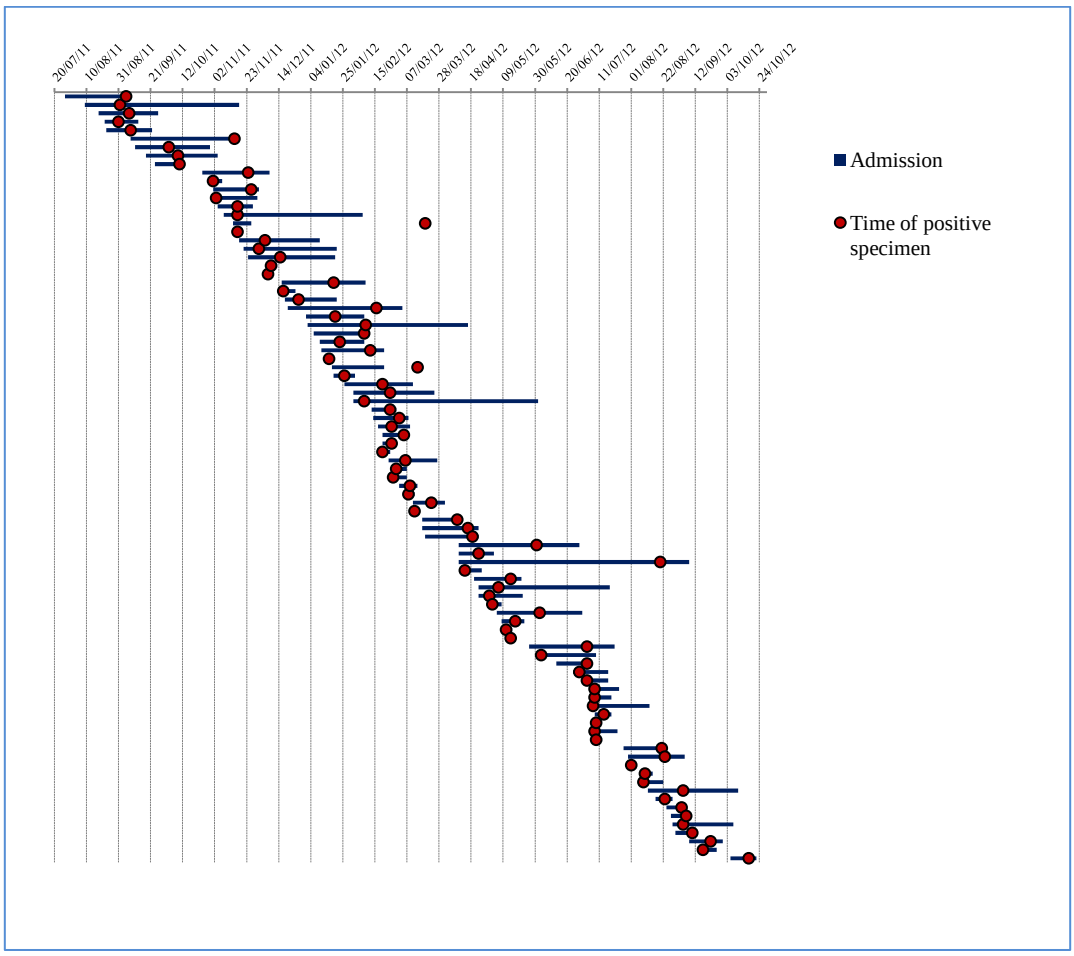


Figure 7: Gantt chart of NDM-1 cases detected from July 2011 to October 2012.

4.1.3 Average length of stay and time to diagnosis

For all potential cases the average time from hospital admission to first detection of NDM-1 was 17.2 days (CI 95% 12.7 – 21.7 days). Potential cases were admitted into an average of 2.5 different wards (CI 95% 2.3 – 2.8 wards) during their stay and had an average hospital stay of 31.2 days (CI 95% 25.5 – 37.0 days; range 1 – 151 days). Of the 83 potential cases which received ICU care the average length of ICU stay was 19.1 days (CI 95% 14.1 – 24.0 days; range 1 – 118 days).

Crude mortality amongst the 86 potential cases was 32.56% (28/86) with the average time from NDM-1 diagnosis to death being 18.8 days (95 % CI 9.28 – 28.3 days).

4.1.4 NDM-1 producing organisms and site of infection

Of the 86 potential cases 51 had invasive disease, namely were not merely colonised with NDM-1 producing Gram-negatives. Figure 8 summarizes the site of infection. For the majority of these cases the primary site of infection was pneumonia (45%, n=23) followed by blood stream infections (35%, n=18), urinary tract infections (14%, n=7) and soft tissue infections (6%, n=3).

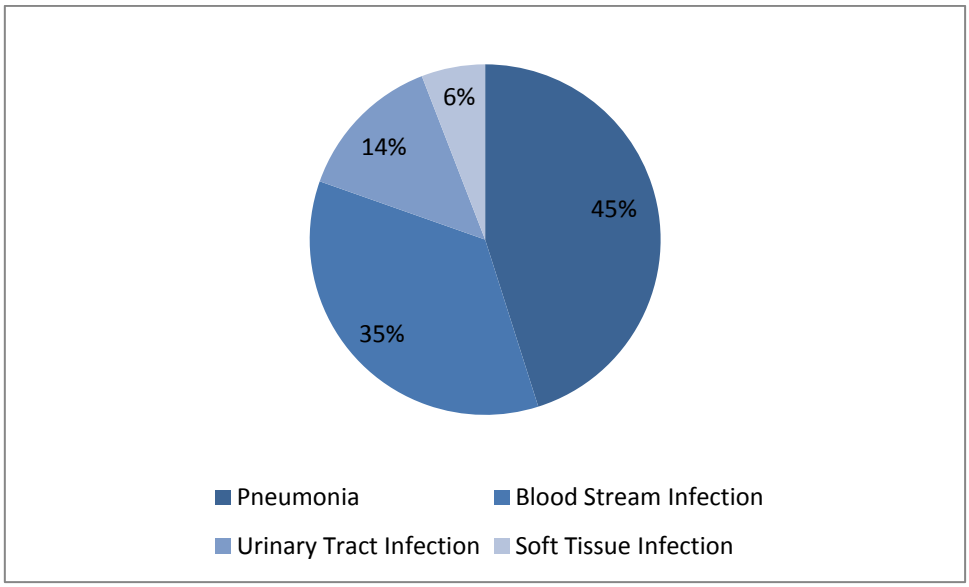


Figure 8: Description of site of NDM-1 infection from outbreak (n=51 from June 2011 to October 2012)

bla$_{\text{NDM-1}}$ was detected on a clinical isolates in 53 of the 86 potential cases, the remaining 33 cases were identified on a dry rectal swab without speciation. Of the 40 potential cases where speciation was done on a clinical isolate, most NDM-1 producers were found to *Klebsiella Pneumoniae* (39/53, 74%), followed by *Enterobacter cloacae* (6/53, 11%), *Serratia*

marcescence (3/53, 6%), *Pseudomonas aeruginosa* (2/53, 4%) and *Klebsiella oxytoca*, *Citrobacter amalonaticus* and *Acinetobacter baumannii* (1/53, 2% each). Figure 9 below provides a summary of these findings.

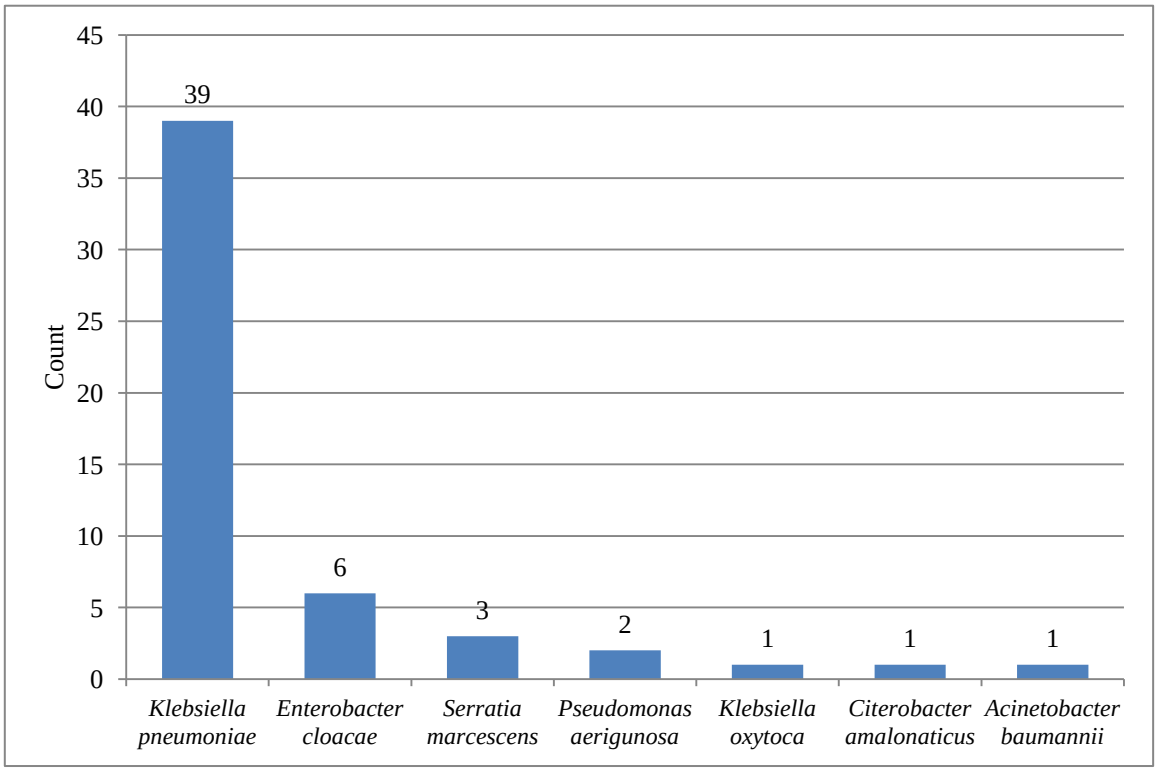


Figure 9: Description of NDM-1 producing isolates from outbreak (n = 53, from June 2011 to October 2012)

4.2 Findings from the case-control study:

4.2.1 Description of cases and controls

The most common NDM-1-producing isolate among the 38 cases included in the case-control study was *Klebsiella pneumoniae* (28/38, 74%) followed by *Enterobacter cloacae* (5/38, 13%), *Klebsiella oxytoca* (2/38, 5%), *Serratia marcescens* (2/38, 5%) and *Citrobacter amalonaticus* (1/38, 3%).

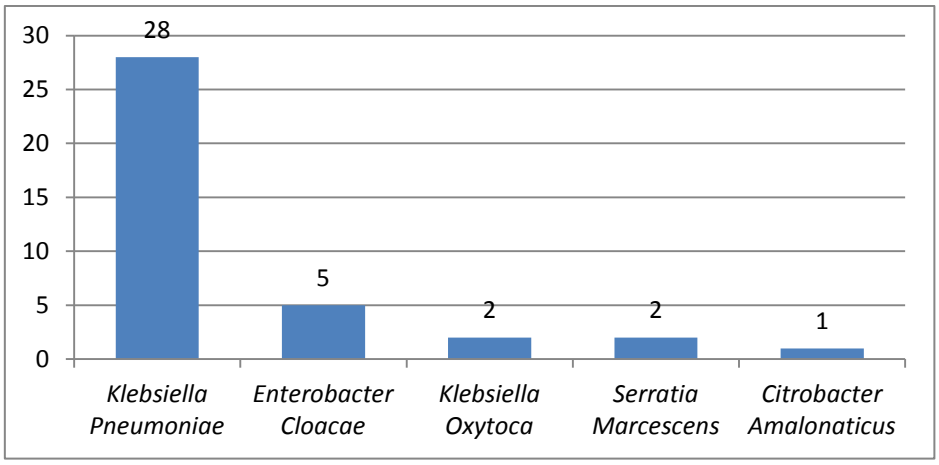


Figure 10: Description of NDM-1 producing isolates

With reference to figure 11, the most common site of infection was lower respiratory tract (20/38, 53%) followed by blood stream infections (13/38, 34%), urinary tract infections (3/38, 8%) and soft tissue infections (2/38, 5%).

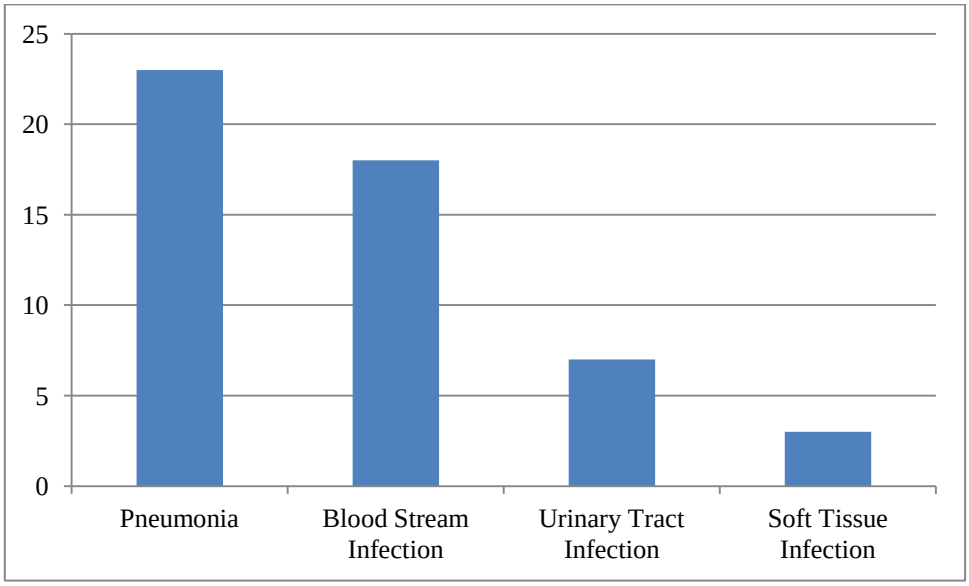


Figure 11: Site of infection with NDM-1 producing Gram negative

As shown in table 8, cases had on average a longer total length of hospital stay (44.0 vs 13.3 days, $P < 0.001$) and longer durations of time at risk, particularly ICU time at risk (18.9 vs 8.3 days, $P < 0.001$) than controls. Charlson co-morbidity index scores were significantly higher in cases than controls (5.2 vs 4.1, P = 0.032).

Table 7: Duration of stay, time at risk and co-morbid status for cases and controls.

Variable	Cases (n=38) Mean (SD)	Controls (n=68) Mean (SD)	*p*-value
Time at risk (total, days)	22.2 (±15.8)	13.3 (±9.5)	**0.004**
Time at risk (intensive care, days)	18.9 (±13.7)	8.3 (±7.2)	**<0.001**
Total length of stay (days)	44.0 (±28.2)	13.3 (±9.5)	**<0.001**
Total length of ICU stay (days)	32.5 (±27.0)	8.3 (±7.2)	**<0.001**
MPM III Score (%)	11.5 (±7.1)	8.3 (±6.8)	0.072
Age Adjusted Charlson Score	5.2 (±3.1)	4.1 (±2.2)	**0.032**

SD = standard deviation; time at risk: from admission to discharge/death (controls) or NDM-1 diagnosis (cases); MPM-III = Mortality Probability Model III; total length of stay: time from admission to discharge/death; *p*-values calculated using Mann-Whitney U test

4.2.2 Factors associated with NDM-1 infection

As shown in Table 9, cases had significantly higher odds of having been hospitalised or admitted to a long-term care facility in the previous year (OR 6.83; 95% CI 2.32 – 20.16) or being transferred from a referral hospital (OR 4.98; 95% CI 1.56 – 15.93) compared to controls.

No association was found between travel history and NDM-1 infection. Although total time at risk was not associated with case status, an ICU stay of longer than seven days was associated with a significant risk of infection with NDM-1-producers (OR 4.82; 95% CI 1.80 – 12.91).

Table 8: Univariate analysis of pre-hospital factors, HIV status, time at risk, surgery and antibiotic exposure among cases and controls.

Exposure Variable	Case patient (n=38) with exposure		Control patient (n=68) with exposure		Unadjusted OR (95%CI)	*p*-value
	Number	%	Number	%		
Previous Hospitalization/Chronic care						
No	10	29	40	83	1	
Yes	24	71	8	17	6.83 (2.32 – 20.16)	**<0.001**
Travel History						
No	30	94	47	98	1	
Yes	2	6	1	2	3.24 (0.29 – 36.63)	0.343
Transfer from referral hospital						
No	23	61	60	88	1	
Yes	15	39	8	12	4.98 (1.56 – 15.93)	**0.007**
HIV Status						
HIV negative	34	89	63	93	1	
HIV positive	4	11	5	7	1.53 (0.29 – 8.11)	0.615
Time at risk (total)						
≤ 14 days	17	45	44	65	1	
> 14 days	21	55	24	35	2.12 (0.97 – 4.62)	0.059
Time at risk (intensive care)						
1 – 7 days	9	24	40	59	1	
>7 days	29	76	28	41	4.82 (1.80 – 12.91)	**0.002**
Surgery*						
No	14	37	33	49	1	
Yes	24	63	35	51	1.60 (0.72 – 3.56)	0.254
Exposure to antibiotics**						
No	5	13	27	40	1	
Yes	33	87	41	60	4.77 (1.38 – 16.48)	**0.014**

*Refers to laparotomy or thoracotomy; **Refers to receiving any dose or either a carbapenem or fluoroquinolone or aminoglycoside or third/fourth generation cephalosporin or piperacillin/tazobactam; OR = odds ratio

Exposure to any antibiotics (carbapenem, fluoroquinolone, aminoglycoside, third- or fourth-generation cephalosporins, or piperacillin/tazobactam) was also significantly associated with case status (OR 4.77; 95% CI 1.38 – 16.48). No association was found between HIV status or surgery (laparotomy or thoracotomy) and infection with NDM-1-producers.

On univariate analysis exposure to aminoglycosides, piperacillin/tazobactam and corticosteroids were significantly associated with case status (Table 10). Each additional dose of piperacillin/tazobactam or a corticosteroid was associated with a 5% increase in odds of developing infection with a NDM-1-producer, while each additional dose of an aminoglycoside was associated with a 3% increase in odds. Although exposure to fluoroquinolones, carbapenems and third-/fourth-generation cephalosporins were associated

with increased odds of case status, none of these showed statistical significance at the 5% level. Each additional day of exposure to a central venous line or indwelling urinary catheter was associated with an 8% and 7% increased odds of case status on univariate analysis respectively. Selected medical interventions were significantly associated with NDM-1-producer infection, with a 16% and 27% increased odds for each additional day of haemodialysis and mechanical ventilation respectively (Table 10).

Table 9: Univariate analysis of exposure to antibiotics, corticosteroids, invasive medical devices and selected medical interventions among cases and controls.

Exposure Variable	Case patient (n=38) with exposure	Control patient (n=68) with exposure	Unadjusted OR (95%CI)	*p*-value
	Mean, (SD)	Mean, (SD)		
Aminoglycosides (dose, any)	10.42 (±22.53)	2.43 (±10.23)	1.03 (1.00 – 1.06)	**0.043**
Gentamycin	0.97 (±5.35)	0.25 (±1.74)	1.07 (0.93 – 1.23)	0.320
Amikacin	7.29 (±18.79)	2.17 (±10.05)	1.02 (0.99 – 1.06)	0.125
Tobramycin	2.16 (±13.30)	0 (±0)	-	-
Fluoroquinolone (dose, any)	1.53 (±3.75)	0.91(±2.76)	1.09 (0.96 – 1.24)	0.162
Ciprofloxacin	0.71 (±3.02)	0.16 (±1.00)	1.19 (0.90 – 1.57)	0.234
Levofloxacin	0.66 (±2.33)	0.49 (±2.32)	1.07 (0.91 – 1.26)	0.429
Moxifloxacin	0.15 (±0.97)	0.26 (±1.32)	0.96 (0.67 – 1.38)	0.830
Carbapenem (dose, any)	16.08(±29.93)	5.59(±11.97)	1.02 (1.00 – 1.05)	0.062
Doripenem	6.16 (±18.43)	0.15(±1.21)	1.18 (0.96 – 1.46)	0.117
Ertapenem	1.39 (±4.03)	1.22 (±3.56)	0.99 (0.88 – 1.12)	0.930
Meropenem	8.52 (±16.74)	4.22 (±11.17)	1.02 (0.99 – 1.05)	0.175
Cephalosporin (dose, any)	2.5 (±7.07)	2.19 (±6.0)	1.00 (0.94 – 1.06)	0.992
Cefepime	1.68 (±6.43)	0.51 (±3.07)	1.06 (0.96 – 1.16)	0.240
Ceftriaxone	0.82 (±2.82)	1.67 (±4.93)	0.93 (0.83 – 1.04)	0.201
Pip-tazobactam (dose)	11.03 (±12.10)	6.17 (±10.31)	1.05 (1.02 – 1.10)	**0.015**
Steroids (dose, any)	23.5 (±23.93)	7.22 (±12.96)	1.05 (1.02 – 1.09)	**0.003**
Invasive Medical Devices				
Central venous line (days)	15.42 (±14.66)	6.51 (±6.71)	1.08 (1.03 – 1.13)	**0.003**
Urinary catheter (days)	18.61 (±15.92)	7.35 (±7.93)	1.07 (1.03 – 1.12)	**0.001**
Medical Interventions				
Mechanical Ventilation (days)	7.47 (±8.55)	0.94 (±2.34)	1.27 (1.10 – 1.48)	**0.001**
Parental Nutrition (days)	2.53 (±3.40)	1.40 (±3.83)	1.07 (0.96 – 1.20)	0.217
Haemodialysis (days)	6.03 (±14.3)	0.68 (±2.74)	1.16 (1.01 – 1.33)	**0.030**

SD = standard deviation; OR = odds ratio.

The final multivariate analysis model showed that having an underlying co-morbid disease as measured by the Charlson co-morbidity index, having had mechanical ventilation and exposure to piperacillin/tazobactam were associated with NDM-1 infection (Table 11).

Table 10: Multiple conditional logistic regression analysis for factors associated with NDM-1 infection

Exposure Variable	Adjusted OR (95% CI)*	*p*-value
Charlson co-morbidity index score	1.59 (1.15 – 2.18)	**0.005**
Mechanical Ventilation (days)	1.32 (1.10 – 1.59)	**0.003**
Piperacillin/tazobactam (dose)	1.08 (1.02 – 1.15)	**0.013**

* Adjusted for Charlson co-morbidity index score, mechanical ventilation and piperacillin/tazobactam; OR = odds ratio

4.2.3 Mortality and excess length of stay associated with NDM-1 infection

Table 12 summarises the findings of mortality and its association with NDM-1 infection. Of the 68 controls, 10 died in hospital (14.7%), while 21 of the 38 cases died in hospital (55.3%).

After adjusting for co-morbid disease, having NDM-1 infection was associated with an eleven-fold higher risk of in-hospital mortality (AOR 11.29; 95% CI 2.57 – 49.60) compared to controls. Cases with bloodstream infections due to NDM-1-producers (AOR 8.84; 95% CI 1.09 – 71.55), or where the organism harbouring the *bla*$_{\text{NDM-1}}$ was *Klebsiella pneumoniae* (AOR 16.57; 95% CI 2.12 – 129.6) had a significantly higher likelihood of in-hospital mortality.

Table 11: Risk factors associated with in-hospital mortality.

Variable	Death (n=31) n (%)	Unadjusted OR (95% CI)	*p*-value	Adjusted* OR (95% CI)	*p*-value
Case - Control					
Control	10 (32)	1		1	
Case	21 (68)	12.81 (2.94 – 55.82)	**0.001**	11.29 (2.57 – 49.60)	**0.001**
Site of Infection					
None	10 (32)	1		1	
Pneumonia	11 (36)	5.5e (-)	0.994	3.54e(-)	0.993
BSI	8 (26)	9.03 (1.10 – 74.21)	**0.041**	8.84 (1.09 – 71.55)	**0.041**
Other	2 (6)	4.37 (0.37 – 51.24)	0.240	3.51 (0.28 – 44.71)	0.333
Isolate					
None	10 (32)	1		1	
K. pneumoniae	16 (52)	19.30 (2.50 – 148.83)	**0.005**	16.57 (2.12 – 129.6)	**0.007**
Other GNB	5 (16)	6.36 (0.72 – 56.51)	0.097	6.08 (0.69 – 53.90)	0.105

*Adjusted for Charlson co-morbidity index; OR = odds ratio; BSI: Blood stream infection; GNB = Gram-negative bacteria

CHAPTER V: DISCUSSION AND LIMITATIONS

5.1 Discussion

This is the largest epidemiological study investigating risk factors and in-hospital mortality associated with NDM-1 infection.[10,11] Further to this, it is the single largest healthcare associated outbreak of NDM-1 producing *Enterobacteriaceae* to date. After a discussion pertaining to the outbreak investigation, key findings from the case control study will be discussed in the context of available literature.

5.1.1 Description of the outbreak

The epidemiologic curve illustrated five clusters of cases with an approximate two week interval between clusters. Each cluster also, typically, had a peak of cases. This is in keeping with a propagating pattern. This pattern is associated with propagated spread, whereby a case is introduced into a susceptible population followed by person-to-person disease transmission. As the number of cases rise there is a concomitant reduction in the number of susceptible patients and the number of new cases decline. A reduction in the number of cases could also be explained by the effects of interventions aimed at halting transmission. During the "inter-peak lull" in cases, the disease is incubating. The incubation period can therefore be estimated as it would be approximately equal to the time difference between these peaks.[84]

In the NDM-1 outbreak setting the epidemiological curve was constructed from all available cases detected during the outbreak, that is to say it includes patients colonised and infected with NDM-1 producing *Enterobacteriaceae.* Incubation refers to the "...interval from receipt of infection to the time of onset of clinical illness"[85] and as colonisation means that the organism is present without causing disease, it would not be possible to estimate incubation periods for NDM-1 producing organisms from this epidemiological curve. However, it was estimated that the average time from admission to detection of NDM-1, either through rectal screening or from molecular testing on a clinical isolate, was approximately 17 days after admission which may inform screening programmes and the index of suspicion for NDM-1 in a clinical setting. Even though the incubation period cannot be estimated from the epidemiological curve, the propagating pattern of the curve strongly suggests person to person spread. The majority of the patients, due to the severity of their illness, were immobile and therefore the most likely route of transmission during this outbreak was via the treating

healthcare workers. During the initial outbreak investigation an environmental sampling study, which included sampling the hands of healthcare workers, was conducted. This study showed that a number of healthcare workers working in the ICU's where the majority of NDM-1 cases were cared for had high levels of contamination on their hands. The environmental sampling also found that a number of ventilation related equipment (e.g. t-piece, suctioning equipment) and stationary (e.g. rulers) were contaminated with NDM-1 producing *K. pneumonia*. Transmission of nosocomial infections and subsequent outbreaks has also been well documented in the literature.[86] The Gantt chart further showed that most of the cases were detected during or subsequent to ICU admission. Therefore it is most likely that the mode of transmission during this outbreak was patient-to-patient via the hands of healthcare workers and contaminated items in the ICU setting.

5.1.1.1 Organisational context of the outbreak

During the outbreak investigation weekly meetings were held between hospital management, clinicians working in the hospital, the primary private laboratory servicing the hospital, infection prevention and control nurse and the NICD outbreak team. There were significant organisational challenges to overcome in effectively addressing the outbreak. The media had run a number of stories on the outbreak and as such hospital management was increasingly concerned about a reputational risk as a result of the prolonged outbreak. Private laboratories held most of the infectious disease and laboratory medicine knowledge. These laboratories, however, service the hospital via doctors requesting laboratory testing with a "preferred laboratory provider". Compensation for services rendered is based on fee-for-service. In this context, the advice from the private laboratories was to institute an expensive PCR based screening programme on dry rectal swab specimens. However, at the time of the outbreak there was no clear evidence to justify using dry rectal swab PCR testing for screening of NDM-1 and subsequent investigations have found the positive predictive value of these tests to be as low as 16%.[87] Therefore the utility of testing needed to be rationalised within the context and resources of the setting, but those with the capacity to inform decision makers in a balanced way were conceivable driven by divergent (profit) interests. Similarly, doctors also operate on a fee-for-service structure and were averse to hospital management implementing measures which may interfere in any way with their clinical decision making. It was also apparent that there was a significant lack in the inter-disciplinary management of patients, often resulting in irrational treatment decisions with patients being on multiple antibiotics prescribed by different physicians. Another example of the lack of inter-

disciplinary care is the fact there were no morbidity and mortality meeting or interdisciplinary ward rounds held at the hospital. In this context, compounded by the fee-for-service and "preferred laboratory provider" enforcing measures to contain the outbreak like patient isolation and cohorting or the rational use of antibiotics, medical devises and ICU beds were exceedingly difficult to achieve. Clinicians are, further to this, not employed by the hospital and typically have a significant information/knowledge advantage compared to managers. It was therefore challenging from an organisational and managerial point of view, to effectively implement measures to reduce transmission and curb the outbreak.

Therefore, it can be argued that current incentive structures, particularly in the private sector, may not be conducive to addressing the drivers of drug resistance and healthcare associated infections namely, reducing the utilization of intensive care, medical devises or antibiotics and may require broader systems reform to be adequately addressed. These incentive structures can result in over-servicing through supply induced demand.[88] Beyond the market failure however, unregulated and over utilization of antibiotics spurs on the insidious rise of drug resistance with significant consequence for the private and the public sector alike. Therefore it is imperative that broader systems consideration be given to change the environment in which clinicians make decisions. A regulatory mechanism through which to achieve this is already provided for in Section 78 of the National Health Act (2004)[89] and the recently established Office of Standards Compliance.[90] The National Core Standards (Domain 2) prioritises Patient Safety, Clinical Governance and Clinical Care and sub-domain 2.6 provides standards for infection prevention and control specifically.[91]

5.1.1.2 Surveillance of carbapenem producing Enterobacteriaceae

As shown earlier in this report, cases of NDM-1 have rapidly spread across the country and to the public sector subsequent to the outbreak. Experience from this NDM-1 outbreak investigation as well as from abroad[56] has shown that once carbapenem resistant *Enterobacteriaceae* become established in a hospital, it is extremely difficult and expensive to eradicate. It is therefore essential that outbreak clusters are detected early through a well functioning surveillance system so that interventions can be put in place to halt spread both within the hospital and the region. This underscores the importance of surveillance which allows for the early identification of cases and in so doing provides an opportunity for reducing the total number of cases and resultant mortality. Findings from this study can be

used to inform a surveillance programme by informing which patients are at greatest risk for NDM-1 infection.

5.1.2 Factors associated with NDM-1 infection

Higher Charlson co-morbidity scores, mechanical ventilation and piperacillin/tazobactam exposure were found to be independent predictors for infection with NDM-1-producers. In-hospital mortality was found to be significantly higher in patients with clinical infection due to NDM-1 producers compared to controls. These findings add evidence to support rational preventive and control measures.

Three previously published papers reporting on risk factors for the acquisitions of NDM-1 in particular were identified. The first was a review of reported cases (n=77) across the European Union which, due to limited data availability, only found travel to India, Pakistan or the Balkans to be associated with NDM-1 acquisition.[92] The second study was a case series (n=5) of a nosocomial outbreak of carbapenem resistant *Enterobacteriaceae* harbouring $bla_{\text{NDM-1}}$ in Canada.[10] The third study, with a cohort design, by Lowe *et al*.[11] investigated nosocomial transmission of NDM-1 to seven patients from two index cases and found exposure to fluoroquinolones, trimethoprim-sulfamethoxazole and carbapenems to be possible risk factors for NDM-1 acquisition.[11] Similar to findings by Lowe *et al*.[11] this study found both carbapenem and fluoroquinolone exposure to be associated, albeit not significantly, with subsequent clinical infection due to a NDM-1-producer. However, trimethoprim-sulfamethoxazole exposure was not assessed in this study as it was not commonly prescribed in the setting of the outbreak. This is the first study to show a significant association between NDM-1 infection and exposure to aminoglycoside and piperacillin/tazobactam.

Findings that an increased duration of exposure to central venous lines, urinary catheters, mechanical ventilation and haemodialysis were associated with an increased risk of infection with NDM-1-producers are consistent with risk factors for the acquisition of carbapenemase-producers identified by previous investigators. For example, studies show that medical devices such as urinary catheters[93,94] and central venous lines[93–95] as well as interventions such as mechanical ventilation[93,95,96] and haemodialysis[93] are well-established risk factors for a range of carbapenemase-producers other than NDM-1. These factors have also been found to increase the risk of acquisition of IMP-type metallo-β-lactamase producing Gram-

negatives.[97,98] This is the first study that identifies and quantifies these exposures for NDM-1-producers.

Of the early NDM-1 cases detected in the United States and United Kingdom, many had epidemiological links to India and Pakistan.[6,67] This present study found no association between international travel and NDM-1 acquisition. Despite not being able to complete the telephonic interview for all cases (32 completed/38, 84%) or controls (48 completed/68, 71%), it is unlikely that international travel was a risk factor for NDM-1 acquisition in the cases linked to this nosocomial outbreak. Of the first five cases identified in the outbreak, none reported any travel history in the year preceding admission, and none of the cases interviewed telephonically reported travel to India, Pakistan or the Balkans, which had been identified as high NDM-1-transmission regions at the time of the outbreak.[6,67] In India, Gram-negative bacteria surveillance isolates collected two years prior to the first identification of NDM-1 has subsequently been shown to harbour $bla_{\text{NDM-1}}$.[99] Similarly, given the lack of standardised surveillance in South Africa, it is likely that $bla_{\text{NDM-1}}$ had been present in clinically-relevant bacteria for some time before the index case was identified.

5.1.3 Mortality and excess length of stay associated with NDM-1 infection

In-hospital mortality for extended-spectrum β-lactamase producers has been reported in other studies at around 37%[100] and amongst patients with carbapenem-resistant *Klebsiella pneumoniae* at between 44% and 48%.[93,96] Crude mortality in patients with bloodstream infections caused by KPC-producing *Klebsiella pneumoniae* is estimated at 53%.[101] Given these reported mortality rates and the limited treatment options available for NDM-1-producers, our finding of a 55.3% crude in-hospital mortality rate was to be expected.

However, considering this outbreak occurred in a well-resourced private sector hospital, mortality rates in patients with similar infections cared for in public sector hospitals in South Africa would be expected to be higher due to limited available antibiotics and ICU facilities. This would likely be the case in many under-resourced healthcare facilities worldwide, which further underscores the importance of taking preventive action to reduce transmission of such multidrug-resistant organisms in the hospital setting, thereby preventing nosocomial outbreaks and limiting dissemination into the community.

5.2 Limitations

Due to the inherent nature of outbreak investigations, there were a limited number of potential cases. All potential cases were reviewed and as many matching controls as were available were included. However, the small sample size limits the study's power to detect other antimicrobial agents as risk factors for infection with NDM-1-producers. The outbreak was confined to the adult ICU, limiting generalisability to a paediatric population. Missing clinical records and missing data on international travel and previous admissions in the year leading up to the admission of interest reduced our sample size and ability to evaluate pre-hospitalization risk factors. The fluctuating point prevalence of NDM-1-producers and the clinicians' enhanced diagnostic suspicion of infection with NDM-1-producers as the outbreak evolved may bias findings. This was, however, addressed by matching controls for date of hospital admission. Information bias could be present in the calculation of the odds ratio's for past admission and travel history as these data were not complete for all cases and controls. Lastly the case-control design limits conclusions on causality.

CHAPTER VI: RECOMMENDATIONS AND CONCLUSION

5.2 Conclusion

NDM-1 infection is associated with significant in-hospital mortality. Risk factors for hospital-associated infection include the presence of co-morbid disease, mechanical ventilation and piperacillin/tazobactam exposure.

Given the dearth of new antimicrobials in the drug development pipeline, the burgeoning threat of conquer by virtually untreatable multidrug-resistant organisms of clinical relevance is becoming realised thanks to the emergence and rapid spread of, amongst others, the carbapenemases.[102,103] Through a better understanding of the risk factors and epidemiological characteristics of patients developing clinical infection with NDM-1-producers, infection prevention and control practice and antimicrobial stewardship programs can be tailored to identify vulnerable patients and prioritise areas for risk reduction, both in an outbreak situation and beyond. This study contributes to a growing body of knowledge for action by identifying risk factors for infection with NDM-1-producers, and highlights the 'bottom line' – such infections exact significant mortality and swift, effective action is needed.

5.3 Recommendations

Based on this report, a number of recommendations are put forward below.

I. Surveillance and screening

Hospital and laboratory based surveillance with obligatory reporting of carbapenem resistant Gram-negatives to the department of health are required. The current NICD surveillance system should be strengthened and capacitated to conduct molecular testing on all isolates reported with possible carbapenem resistance. Surveillance reports should be communicated to various stakeholders including the National and provincial departments of health as well as private sector hospitals so that appropriate action can be taken in timeous manner.

National guidelines on screening for NDM-1 and other carbapenem resistant *Enterobacteriaceae* need to be developed and frequently revised based on the findings from this investigation, other studies and future findings. From this study screening should be targeted at ICU patients with extended stays of more than a week; with medical devises in

situ, particularly those requiring mechanical ventilation. Patients receiving antibiotics, particularly piperacillin/tazobactam, an aminoglycoside or a carbapenem should also be included in a screening programme.

II. Infection prevention and control

In-hospital spread needs to be reduced with a particular focus on hand hygiene. As noted in this report, the outbreak was probably spread from person-to-person via healthcare workers in the ICU setting. Hand hygiene needs to be taught and practiced by all healthcare workers and management must ensure easy access to appropriately equipped hand washing stations.

Secondly, patients found to be harbouring NDM-1 producing *Enterobacteriaceae* must be isolated and strict contact precautions must be maintained in order to reduce transmission to other patients and protect healthcare workers from being colonised with NDM-1 producing organisms.

Thirdly, the utilization of ICU care and invasive medical devises must be rationalised. As shown in this study increased hospital and particularly ICU stay significantly increases risk of infection with NDM-1 producing *Enterobacteriaceae*. Further it was shown that medical devises are associated with acquisition of NDM-1. Therefore it is recommended that facilities as well as provincial/national department of health develop guidelines for ICU admission in an effort to reduce the utilization and possible exposure to drug resistant organisms. Further, guidelines on invasive medical devises should be developed and infection prevention and control practitioners should be equipped to monitor the utilization of devises and alert treating physicians if and when devices have been in situ for extended periods of time and require removal/replacement.

III. Antibiotic stewardship

As demonstrated in this study, exposure to antibiotics is an important risk factor associated with NDM-1 infection. Therefore, an essential component of addressing resistance is instituting antibiotic stewardship programmes at a national, sub-national and most importantly an institutional level. Antibiotic stewardship requires a multidisciplinary approach (clinicians, pharmacologists, managers, microbiologists and infection prevention and control specialists) to design, update and implement. It pertains to the utilization of the correct antimicrobial agent at the correct dose for the correct duration taking into account

possible selective pressures for resistance and drug toxicity.[104] Therefore, antibiotic stewardship programmes require local epidemiological and resistance pattern data to be effective and relevant. Their effectiveness and relevance also depends on a continuous reassessment of evidence, practice and changing epidemiology. Forums such as multidisciplinary morbidity and mortality meeting should be leveraged and strengthened as platforms for developing and implementing antibiotic stewardship programmes.

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Copy of Ethics Clearance Certificate

R14/49 Dr Pieter de Jager

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130248

NAME: (Principal Investigator)	Dr Pieter de Jager
DEPARTMENT:	School of Public Health Medical School
PROJECT TITLE:	An Outbreak of New Delhi MetalloB-lactamase 1(NDM) Producing Enterobacteriaceae in a South African Hospital: A Case Control Study
DATE CONSIDERED:	22/02/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Juno Thomas
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 24/05/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES