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Investigation of carbapenem-resistant *Enterobacterales* isolates at a tertiary laboratory in Pretoria, South Africa

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RESEARCH ARTICLE



ABSTRACT

This study aimed to investigate phenotypic and genotypic characteristics of carbapenem-resistant *Enterobacterales* isolates (CRE) at a tertiary laboratory in South Africa. A total of 99 CRE isolates were collected between 2019 and 2021. Carbapenemase production was tested using modified carbapenem inhibitory method. Colistin susceptibility testing was performed using the ComASP™ Colistin broth microdilution method. Conventional PCR assays were conducted for detection of *mcr-1* gene and common carbapenemase genes (*bla*_{VIM}, *bla*_{NDM}, *bla*_{IMP}, *bla*_{KPC}, *bla*_{OXA-23}, *bla*_{OXA-51} and *bla*_{OXA-48}). Rep-PCR assay was conducted to determine the genetic relatedness of the study isolates. Majority of the isolates were *Klebsiella pneumoniae* (83%). Carbapenem resistant *K. pneumoniae* cluster was observed from ICU and surgical ward samples. Colistin resistance was observed in 13% (12/93) of the isolates namely, in 11 *K. pneumoniae* and one *Enterobacter cloacae*. The *bla*_{OXA-48} (65%) was the most prevalent gene detected followed by *bla*_{NDM} (25%) and *bla*_{VIM} (22%). Several *K. pneumoniae* isolates concomitantly carried multiple carbapenemase genes with one isolate carry up to 5 five genes *bla*_{VIM}, *bla*_{NDM}, *bla*_{OXA-48}, *bla*_{OXA-23} and *bla*_{OXA-51}. The *mcr-1* gene was not detected in the isolates. Rep-PCR assay showed that most isolates matched cluster A (50%). The high prevalence of *bla*_{OXA-48}, *bla*_{NDM} and emerging colistin resistant isolates is of concern for patient management at this institution and needs close monitoring. Rep-PCR is a valuable tool in establishing infection clusters in resource-limited settings.

KEYWORDS

carbapenem, carbapenem resistant *Enterobacterales*, antimicrobial resistance, *Klebsiella pneumoniae*, ICU

INTRODUCTION

Antimicrobial resistance (AMR) has become a global health threat [1]. Antimicrobial resistance is associated with increased morbidity and mortality, as well as higher healthcare costs [1]. The increasing burden of AMR is mostly caused by inappropriate use of antimicrobials and poor infection prevention and control (IPC) practices in healthcare facilities [1]. Carbapenem-resistant *Enterobacterales* (CRE) isolates are usually multidrug-resistant (MDR) or extensively drug-resistant (XDR); which makes the infections caused by CRE a healthcare challenge [2]. CRE causes a wide range of infections from self-limiting to life-threatening diseases including bloodstream infections, urinary tract infections (UTIs), skin and soft tissue infections, and pneumonia [2].

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Treatment options for CRE infections include combination therapy with polymyxins (polymyxin B and colistin), fosfomycin, tigecycline, and aminoglycosides [3]. Colistin is currently the most used monotherapy as a last resort to treat CRE infections [3]. However, the development of resistance through acquisition of plasmids such as the mobilized colistin resistance genes (*mcr*) is of concern [3]. There are several concerns with using these agents such as dosing uncertainties, limited efficacy, toxicity, and the development of resistance [4]. Older antimicrobials such as doxycycline, minocycline, chloramphenicol, and trimethoprim/sulfamethoxazole may be effective for some CRE isolates [5]. The proportion of antimicrobial resistant *Enterobacterales* family has increased globally, leaving clinicians with limited treatment options for serious infections caused by these organisms [3]. This poses a major threat to infectious disease treatment and the control of infections within healthcare facilities [6].

Carbapenem resistance is primarily mediated by carbapenemases, which are enzymes that inactivate carbapenem antimicrobials [3]. Carbapenemases are classified into Ambler class A or D serine β -lactamases and Ambler class B metallo- β -lactamases (MBLs) [3]. Carbapenem resistance is commonly conferred by enzymes such as *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo- β -lactamase (NDM) [7]. Other carbapenemase genes such as *bla*_{IMP}, *bla*_{VIM}, and *bla*_{OXA-48} have also been detected in carbapenemase-producing *Enterobacterales* (CPE) [3]. Carbapenemase genes are transported from one host to another through transposons and plasmids which facilitate the development of outbreaks in healthcare settings [3]. The rapid increase and dissemination of AMR genes within healthcare facilities indicate the need for constant updates on circulating strain genetic diversity. Therefore, this study aimed to investigate the phenotypic and genotypic characteristics of CRE isolates as well as their genetic relatedness. Knowing the genetic relatedness of the isolates will assist with advising IPC practices as most of these organisms are known to cause outbreaks in hospital settings [1].

MATERIALS AND METHODS

Ethical approval, study design, setting and sample collection

The study was approved by Sefako Makgatho Health Sciences University Research Ethics Committee (SMUREC/M/249/2020: PG), and permission to collect study isolates was granted by the Microbiology Dr George Mukhari Tertiary Laboratory (DGMTL) manager prior to samples collection. A total of 99 clinical isolates were collected between 2019 and 2021 at DGMTL, a unit of the National Health Laboratory Service (NHLS) of South Africa after routine diagnostic testing. The laboratory primarily serves Dr George Mukhari Academic Hospital (DGMAH), 3 district hospitals and other surrounding primary healthcare facilities. The isolates were transported to the Microbiological Pathology

Department, Postgraduate Research Laboratory at Sefako Makgatho Health Sciences University for processing. Isolates were sub-cultured, and a pure colony of each isolate was stored in Microbanks[®] (Pro-Lab Diagnostics, Canada) at -20°C for future use.

Bacterial identification and antimicrobial susceptibility testing

Bacterial identification and antimicrobial susceptibility testing on the isolates were performed using VITEK[®]-2 systems (bioMérieux, France). Isolates that were reported as carbapenem-resistant from VITEK[®]-2 systems (bioMérieux, France) were phenotypically tested for carbapenemase production using the modified carbapenem inhibitory method (mCIM). Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [8]. *K. pneumoniae* ATCC BAA1705 and *K. pneumoniae* ATCC BAA 1706 were used as positive and negative controls for mCIM, respectively.

Confirmation of colistin susceptibility results

Colistin susceptibility testing results were confirmed by the broth microdilution method using the ComASP[™] Colistin broth microdilution (Liofilchem[®] srl, Diagnostic, Italy). Briefly, bacteria preserved in Microbanks were inoculated on the Mueller Hinton agar plates and incubated at 37°C for 18–24 h to allow for growth. A loopful of the colony was suspended into a microcentrifuge tube with 0.9% saline (DMP-NHLS, South Africa) to prepare a standardized inoculum to the density of 0.5 McFarland. The adjusted suspension was diluted to 1:20 in saline (solution A). A total of 0.4 ml of solution A was added to a tube of Mueller Hinton broth contained in the kit (Solution B). A hundred microliters of solution B was dispensed into each well in a row containing colistin antibiotic. The panel was covered with a lid provided and incubated at 37°C for 16–20 h in ambient air. Results were read by two blinded observers visually (naked eyes) in bright light against a dark background. *Escherichia coli* NCTC 13846 and *Pseudomonas aeruginosa* ATCC 27853 were used as positive and negative controls for colistin broth microdilution, respectively.

PCR assays for genomic confirmation of *K. pneumoniae* isolates identification

The total genomic DNA of isolates was extracted using the boiling method. A conventional PCR assay was performed for the confirmation of *K. pneumoniae* targeting the *phoE* housekeeping gene [9]. All the primers used in this study were synthesised by Inqaba Biotechnical Industries (Pty) Ltd, South Africa, and are presented in Table 1. PCR assays in this study were conducted in a Bio-Rad thermo-cycler (Bio-Rad, USA).

Molecular detection of *mcr-1* in *K. pneumoniae* isolates

The *mcr-1* gene was screened in the isolates by PCR assay with conditions previously described [10] (Table 1).



Table 1. Primers used for conventional PCR

Genes	Primer name	Sequence direction (5'→3')	Size (bp)	Reference
<i>K. pneumoniae</i> Identification (<i>PhoE</i>)	PhoE-F	GTGCGATGCGGTCTTTG	398	[9]
	PhoE -R	GGGCGAACTGAACTGATG		
Colistin resistance gene <i>mcr-1</i>	mcr-1_F	CGGTCAGTCCGTTTGTTC	309	[10]
	mcr-1_R	CTTGGTCGGTCTGTAGGG		
Carbapenemase genes	<i>bla</i> _{KPC}	KPC_F	893	[11]
		KPC_R		
	<i>bla</i> _{IMP}	IMP-F	139	[12]
		IMP-R		
	<i>bla</i> _{VIM}	VIM-F	390	[12]
		VIM-R		
	<i>bla</i> _{NDM}	NDM-F	129	[13]
		NDM-R		
	<i>bla</i> _{OXA-23}	OXA-23_F	501	[14]
		OXA-23_R		
	<i>bla</i> _{OXA-48}	OXA-48_F	438	[14]
		OXA-48_R		
	<i>bla</i> _{OXA-51}	OXA-51_F	353	[14]
		OXA-51_R		
Rep-PCR	Rep-PCR_F	IIIGCGCCGICATCAGGC		[15]
	Rep-PCR_R	ACGCTTATCAGGCCTAC		

K. pneumoniae ATCC 1705 strain and nuclease-free water were used as positive and negative controls, respectively.

Molecular detection of CRE genes in *K. pneumoniae* isolates

Multiplex and simplex PCR assays were performed for the detection of common carbapenemase genes *bla*_{VIM}, *bla*_{NDM}, *bla*_{IMP}, *bla*_{OXA-23}, *bla*_{OXA-48}, *bla*_{OXA-51} and *bla*_{KPC} [11–14] (Table 1).

Visualisation of PCR products

The PCR products were separated on a 2% (*m/v*) agarose gel (Bioline, Taunton, MA, USA). Gel electrophoresis was performed at 100 V for 60 min. A 100 bp molecular weight marker (Fermentas, Thermo Scientific, Waltham, MA, USA) was included as a reference in all gels. The gel images were visualised and captured using a Gel DocTM EZ system (Bio-Rad, Hercules, CA, USA).

Repetitive element palindromic PCR (Rep-PCR) for *K. pneumoniae* isolates

Rep-PCR was used on selected CRE isolates to determine the genetic relatedness of the isolates at DGMA hospital. It was performed on *K. pneumoniae* isolates from ICU and surgical wards of DGMA hospital because they had a high number of isolates. REP regions were amplified according to protocol previously reported [15] using MyTaqTM HS Red Mix (Bioline, UK) (Table 1). The results of the Rep-PCR were grouped as clades according to their matching patterns as previously described [15]. The dendrogram of *K. pneumoniae* isolates was constructed from the banding

patterns using the PyElph analysis software version 1.4; and the clustering method was applied on the distance matrix, then the relatedness was shown by means of the unweighted pair group method with arithmetic mean (UPGMA).

RESULTS

Distribution of CRE isolates from different wards

A total of 99 CRE isolates were collected from DGMTL after routine diagnostic tests. Most isolates were from ICU 29% (29/99) followed by surgical ward 10% (10/99). Among the CRE isolated, *K. pneumoniae* was most prevalent with 82 isolates followed by *Enterobacter cloacae* complex with 6 isolates. Figure 1 illustrates Carbapenem-resistant *Enterobacterales* identified by the VITEK[®]-2 system.

Antimicrobial susceptibility profiles of CRE isolates

The susceptibility profiles of CRE isolates showed a high level of antimicrobial resistance among the carbapenems, of which ertapenem, meropenem and imipenem resistance was at 87, 76 and 68% respectively. The CRE isolates also revealed a high rate of resistance to non-β-lactams such as gentamicin and ciprofloxacin at 81 and 71%, respectively; however, among the non-β-lactams, amikacin showed the high rate of susceptibility at 82%. The complete antimicrobial susceptibility patterns of the CRE isolates tested using the VITEK[®]-2 system are presented in Fig. 2. Colistin susceptibility was further established using the ComASPTM Colistin broth microdilution.



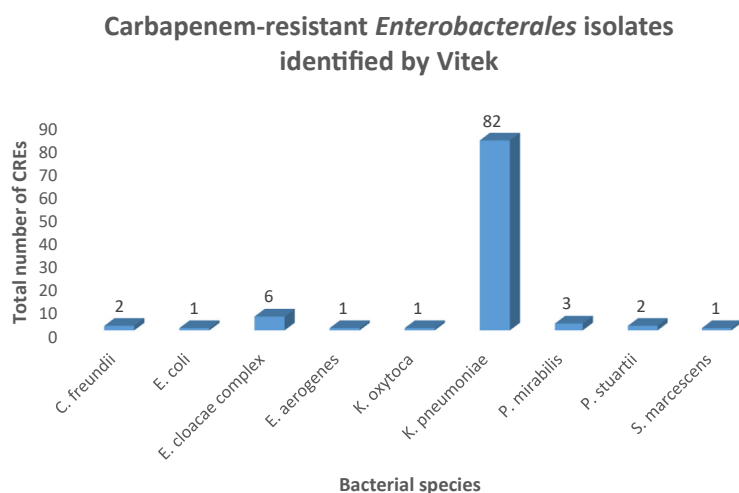


Fig. 1. Species distribution of isolates identified by VITEK[®]-2 system. *K. pneumoniae* was the most prevalent among the CRE isolates

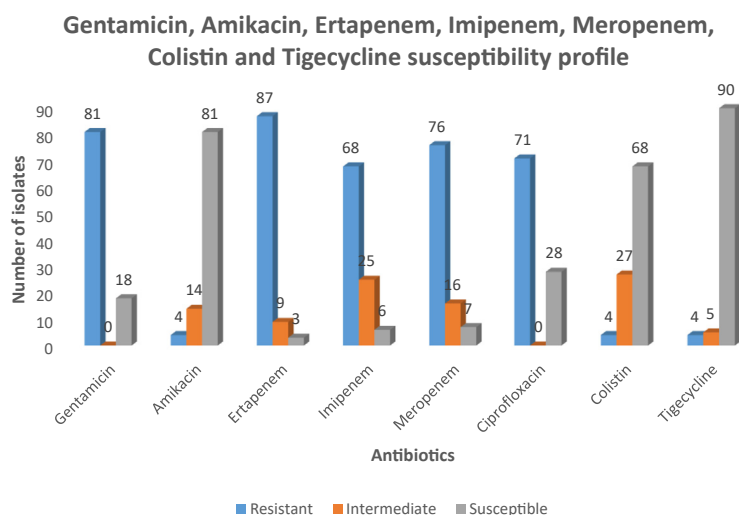


Fig. 2. Antimicrobial susceptibility profile of all CRE isolates by VITEK-2 system

Colistin broth microdilution susceptibility profiles of the study isolates

ComASP[™] Colistin broth microdilution results were viewed at the end of the incubation period; growth was observed in the wells and the MIC was established. Growth appeared as turbidity at the bottom of the well. Colistin susceptibility profile was conducted on 93 out of 99 study isolates, with isolates intrinsically resistant to colistin ($n = 6$) were excluded, that had initially been investigated by VITEK-2. From the 93 tested isolates, 11 (12%) *K. pneumoniae* were resistant to colistin and 71 isolates (76%) were intermediate. One *E. cloacae* complex was resistant to colistin with 5 isolates being intermediate.

Molecular detection of antimicrobial resistance genes of CRE isolates

From the 99 CRE isolates collected, *bla*_{OXA-48} was the most prevalent gene 65% (64/99) followed by *bla*_{NDM} with 25%

(25/99) positive isolates and *bla*_{VIM} with 22% (22/99) positive isolates. Both *bla*_{IMP} and *bla*_{KPC} genes were not detected among the isolates. The *bla*_{OXA-48} gene was detected in 70% (57/82), the *bla*_{NDM} in 24% (20/82) and the *bla*_{VIM} in 22% (18/82) of *K. pneumoniae* isolates (Fig. 3).

Out of 99 CRE isolates tested, 45 isolates were positive for only 1 carbapenemase gene and 54 isolates were positive for more than 1 gene. Among the 54 isolates harbouring multiple genes, most isolates were *K. pneumoniae*. The study further investigated the profiles of *K. pneumoniae* isolates and found that 24 isolates carried 2 genes each with 2 isolates harboring *bla*_{VIM} and *bla*_{NDM}, 9 isolates harboring *bla*_{VIM} and *bla*_{OXA-48} and 13 isolates harbored *bla*_{NDM} and *bla*_{OXA-48}. Two *K. pneumoniae* isolates were positive for 3 genes where 1 isolate had a combination of *bla*_{VIM}, *bla*_{OXA-23} and *bla*_{OXA-51} and another isolate had *bla*_{OXA-48}, *bla*_{OXA-23}, and *bla*_{OXA-51}. Interestingly, one isolate was positive for 4 genes which was the combination of *bla*_{VIM}, *bla*_{NDM}, *bla*_{OXA-}

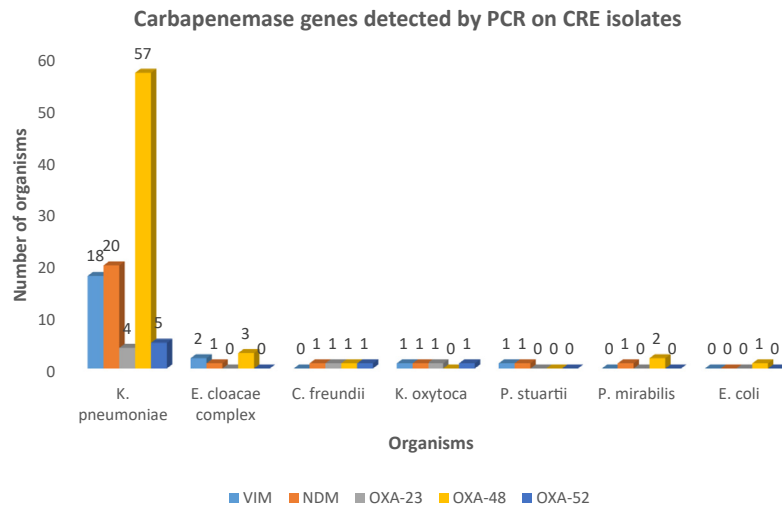


Fig. 3. Carbapenemase genes detected by PCR. The *bla*_{OXA-48} and *bla*_{NDM} were the most prevalent carbapenemase genes in this study

23 and *bla*_{OXA-51}. Another isolate had the combination of 5 genes which were *bla*_{VIM}, *bla*_{NDM}, *bla*_{OXA-48}, *bla*_{OXA-23} and *bla*_{OXA-51}. Figure 4 illustrates the multiple carbapenem resistant genes detected among *K. pneumoniae* isolates.

Molecular typing of CRE isolates

The Rep-PCR was used to derive clonality of the isolates. The results from the constructed dendrogram (Fig. 5) showed 3 main clusters; A (0042, 0401, 0699, 3159, 3551, 3607, 3940, 5328 and 6975 and 9577), B (6087 and 6246) and C (3012, 7078, 7206, 7222, 9110 and 9550). The rest of isolates were unrelated and categorised as singletons, while three isolates (3270, 0109 and 0256) were untypeable by Rep-PCR (data not shown on the dendrogram).

DISCUSSION

The increasing prevalence of MDR Gram-negative bacteria has become a serious threat to public health worldwide [3].

There is lack of effective antimicrobial agents to treat infections caused by these organisms in hospital settings [3]. Carbapenems have long been thought to be the most effective antimicrobials for treating MDR Gram-negative bacteria that cause serious infections. However, the ever-increasing incidence of carbapenemase gene carriage by *Enterobacterales* has considerably reduced their potency. The aim of this study was to investigate phenotypic and genotypic characteristics of CRE isolates at DGMTH.

Patients infected with CREs, as well as CRE carriers, play a key role in CRE transmission in healthcare facilities and are at a high risk of acquiring infections caused by multi-drug-resistant bacteria [16]. In our study, the highest number of CRE isolates were *K. pneumoniae* at 83% followed by *E. cloacae* complex at 6% and *Proteus mirabilis* at 3%. These findings are similar to the findings reported by Li et al. in 2018 [7] where carbapenem-resistant *K. pneumoniae* (CRKP) were the most common species, accounting for 52.9% followed by *E. coli* at 17.5% CRE isolates [7]. In 2016, a Turkish study also reported similar results from a tertiary

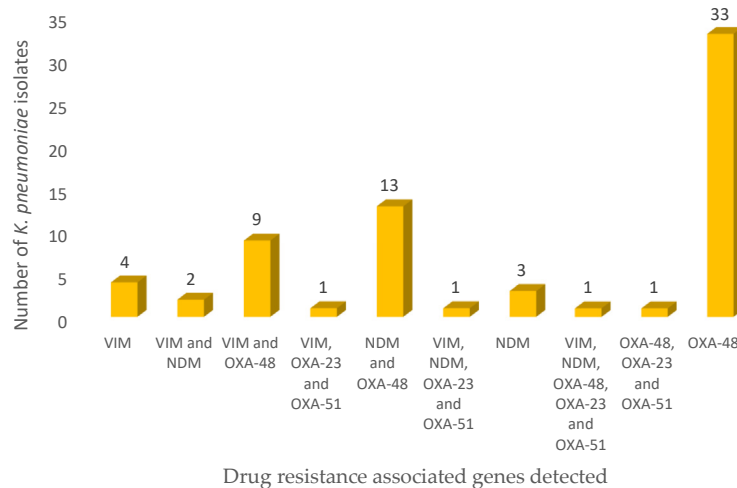


Fig. 4. Combinations of carbapenem resistance genes in *K. pneumoniae* isolates



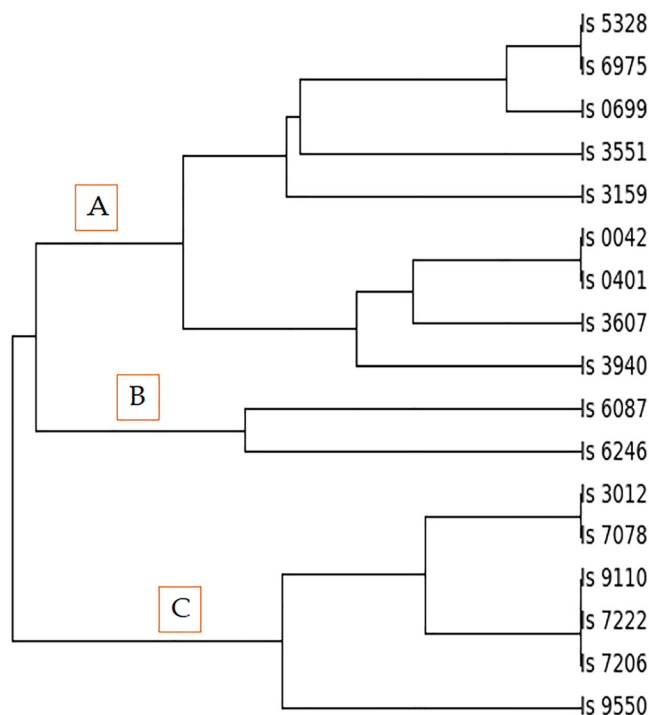


Fig. 5. The dendrogram constructed using PyElph analysis software version 1.4 from the banding patterns obtained after REP-PCR of a representative number of *K. pneumoniae* isolates. The dendrogram revealed genetic relatedness of the isolates which belonged to three main clusters (A, B, C)

level-reference hospital, where the most common species were *K. pneumoniae* 69 (38.12%), followed by *Serratia marcescens* 33 (18.23%), and *Morganella morganii* 17 (9.39%) [17]. This indicates that the leading species circulating in hospitals causing CRE infections is *K. pneumoniae* globally [18].

Patients in intensive care units (ICU) are particularly at high risk of developing bacterial infections caused by MDR strains [18]. The development and high incidence of CRE pose a serious threat to these individuals. In the present study, the overall prevalence of *K. pneumoniae* was 83% (82/99) in all the wards which was the highest among the collected CRE isolates. Twenty-four out of eighty-two (29%) of these appear to have been isolated from ICU which is higher than CRKP isolated from other wards. In China, Li et al., reported similar findings in 2018, where the overall prevalence of carbapenem resistance among the *K. pneumoniae* isolates was (36.2%), regardless of the department [7]. However, 57.1% of *K. pneumoniae* isolates from the ICU were found to be carbapenem resistant, which was significantly higher than the proportions from the surgical ward, medical ward, paediatric ward, and other departments. These findings emphasize that patients in ICU are at an increased risk of developing bacterial infections caused by CREs. This may be due to cross infection due to a bridge in infection prevention and control measures. There might have also been an outbreak of CRKP as suggested by the clustering of isolates shown by Rep-PCR which should be

investigated in future studies to advise on measures that need to be put in place to reduce the likelihood of this occurring again.

Susceptibility profiles of all isolates in this study showed a high-level resistance to carbapenems such as ertapenem (88%), meropenem (77%) and imipenem (69%), respectively. A Chinese study by Hameed and colleagues reported resistance in the study isolates against the ertapenem, imipenem, meropenem, tigecycline and colistin to be 87.5%, 69%, 78.8%, 7% and 43.2% respectively [19]; which support the findings in this study. The isolates in this study showed a high rate of resistance to non- β -lactams such as gentamicin and ciprofloxacin at 82% and 72% respectively with 82% amikacin susceptible isolates. Dilagui et al. in 2022 reported the CRE high co-resistance rate to other non- β -lactam antibiotic classes and more than 94% of isolates were susceptible to amikacin [20]. The high rate of amikacin susceptible CRE isolates means that amikacin can be used an alternative drug for treatment of CRE infections [20]. Another study by Taha et al. in 2023 reported a high resistance rate of CRE isolates to gentamicin, amikacin and ciprofloxacin [21].

Four isolates each were resistant to colistin and tigecycline; and among them three were intrinsically resistant to colistin according to VITEK[®]-2 system. A total of 69% were susceptible to colistin. The emergence of colistin resistance shows that there may not be any treatment options available to manage CRE infections soon for settings where newer agents like ceftazidime-Avibactam and Aztreonam are not accessible [22].

This study detected colistin resistance in 11 of the *K. pneumoniae* isolates using the ComASP[™] Colistin broth microdilution test while 71 *K. pneumoniae* isolates were found to have intermediate resistance to colistin. This is alarming as colistin is considered the last resort of treatment for infections caused by CRE. Five *E. cloacae* complex were found to have intermediate colistin resistance while 1 isolate was colistin-resistant. Resistance was observed only in two organisms, *K. pneumoniae* and *E. cloacae* complex with the remainder of the organisms being intermediately resistant to colistin. A study by Kar et al., 2021 reported colistin resistance detected in 27 (13%, 27/200) CRE isolates as per the reference broth microdilution method, and the majority (22/27, 81%) were *K. pneumoniae*, followed by *E. coli* (3/27, 11%) [23].

The most detected carbapenemase gene in this study was *bla*_{OXA-48} at 65% of all isolates. The *bla*_{NDM} gene was detected in 25% followed by *bla*_{VIM} in 22% of all isolates. A previous report indicated that the two main carbapenemase enzymes currently detected in South Africa are *bla*_{OXA-48} and *bla*_{NDM} [24]. According to a report by GERMS-SA: Annual Surveillance Review 2019, compiled by the National Institute of Communicable Diseases (NICD), from four provinces in South Africa, the *bla*_{NDM} gene was detected in 29% and *bla*_{OXA-48} in 61% of blood culture samples which were received as part of surveillance testing [24]. Since most isolates detected in this study were *K. pneumoniae*, the genes detected were further analysed among the *K. pneumoniae* isolates. From



the *K. pneumoniae* isolates, 70% (57/82) harboured the *bla*_{OXA-48} gene, followed by 24% (20/82) *bla*_{NDM} and 22% (18/82) *bla*_{VIM}. A study by Meng et al., 2023 reported a consistent increasing trend of *bla*_{OXA-48} CHβLs in *K. pneumoniae* globally [25]. Of concern also is the isolates harbouring *bla*_{NDM}, this has a major implication on treatment options with newer agents' ceftazidime-avibactam not being active against these isolate. The increasing identification of such isolate stresses the need for other agents like aztreonam to be made more available which is not the case in resource constrained institutions. The *bla*_{IMP} and *bla*_{KPC} genes were not expressed in any of the isolates, this may be because KPC-producing organisms are prevalent in North America and Europe and result in sporadic outbreaks in other parts of the world [7].

Carbapenemase-producing *Enterobacterales* have recently emerged, and several case reports and studies have been published globally [26]. Multiple carbapenemase genes-carrying isolates are difficult to eliminate and are becoming a significant concern owing to the likelihood of global spread [26]. Our study detected 29% of *K. pneumoniae* isolates which carried 2 carbapenemase genes, 2% of isolates with 3 genes and 1% each with 4 and 5 carbapenemase genes. A similar observation has previously been reported by Kilic and Baysallar, 2015 in Turkey, who reported the co-harboring of *bla*_{NDM-1} and *bla*_{OXA-48} in one *K. pneumoniae* isolate [27]. These findings pose a threat to hospital settings since isolates with multiple carbapenemase genes are difficult to manage and can disseminate globally. Infection prevention and control measures should be put in place and reinforced to minimize the spread of these infections. There are limited effective treatment options for the management of CRKP therefore, preventing their spread in healthcare settings like ICUs is critical [28]. Hand hygiene, contact precautions and isolation are required for patients found to be infected or colonized with CREs [28].

Enterobacterales are the primary hosts of the *mcr-1* gene, which was discovered in *E. coli* and subsequently transferred to other bacteria such as *K. pneumoniae*, causing many epidemics [7]. In the present study, 12 isolates were found to be resistant to colistin following MIC determination by the ComASP™ Colistin broth microdilution. The resistant isolates were further subjected to conventional PCR for detection of the *mcr-1* gene. However, the gene was not detected. This could be due to other mechanisms of colistin resistance. Further studies should be conducted for further investigation of mechanisms of colistin resistance in these isolates.

In the present study, Rep-PCR was used to determine the genetic relatedness of the 30 *K. pneumoniae* isolates (24 from ICU and 6 from surgical ward). The results of banding patterns obtained after Rep-PCR analysis have shown close genetic similarity between isolates collected from ICU and surgical ward on different dates, which were divided into five clades sharing the same genetic characteristics. Isolate 3607 from ICU (collected on the 07/03/2019) was similar to isolate 2948 from surgical ward (collected on the 12/02/2020); also, isolate 2128 from surgical ward (collected on the 09/02/2020) was similar to

both isolates 4779 (ICU) collected on the 03/12/2019 and isolate 9577 (ICU) collected on the 13/05/2021 indicating that the strains could have moved to the subsequent ward when the patient was discharged from ICU to a general ward. This is worrying since closely related isolates are circulating between the ICU and other wards of the Hospital.

The results have shown that Rep-PCR is still a useful genotyping technique to determine the genetic relatedness of isolates in resource-limited settings. It can facilitate outbreak detection and investigation and reveal common sources of infection in conjunction with epidemiological investigations [29]. Whole genome sequencing in contrast to Rep-PCR used in our study is considered the gold standard for molecular typing and has proven to be fast, reliable and can provide increased genetic information, including antibiotic resistance genes, virulence factors and sequence types of *K. pneumoniae*. However, the high cost has limited its routine implementation [30]. A similar study conducted in Iran has shown Rep-PCR to be a considerably suitable tool for the rapid identification of strains with high epidemic potential that may be required for local outbreak studies in resource-limited settings [29].

CONCLUSIONS

Carbapenem-resistant *K. pneumoniae* was the most common CRE organism identified in this study. Carbapenem-resistant *Enterobacterales* isolates harboring multiple carbapenemase genes are a growing concern at DGMAH. The carbapenemase gene *bla*_{OXA-48} was the most prevalent gene detected followed by *bla*_{NDM} in this study. Colistin resistance is starting to emerge which poses a serious threat to the management options for patients. The study findings showed that Rep-PCR may be a useful method for the rapid characterization of isolates with high outbreak potential in resource-limited settings.

Author contributions: Conceptualization, M.R.; T.D.P. and A.M.M.; methodology, M.R. and G.M.N.; software, M.R. and G.M.N.; validation, N-D.N., A.M.M and T.D.P.; formal analysis, N-D.N., M.R. and G.M.N.; investigation, M.R., G.M.N. and N-D.N.; resources, M.N and A.M.M.; data curation, J.Y.B, A.M.M and T.D.P.; writing—original draft preparation, M.R. and G.M.N.; writing—review and editing, N-D.N., J.Y.B. T.D.P., M.N. and A.M.M; visualization, M.R. and G.M.N.; supervision, T.D.P. and A.M.M.; project administration, A.M.M and T.D.P.; funding acquisition, A.M.M and M.N. All authors have read and agreed to the published version of the manuscript.

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