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Original Research Article

Phenotypic detection of extended-spectrum beta-lactamase produced by *Klebsiella pneumoniae* isolated from different clinical samples from intensive care unit, Khamis Military Hospital, Abha, Kingdom of Saudi Arabia

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ABSTRACT

Background: *Klebsiella pneumoniae* is an opportunistic pathogen that is one of the commonest causes of infection in intensive care units. *K. pneumoniae* infection have contributed considerably to morbidity and mortality in patients with chronic illnesses. The coexistence of virulence factors and the genetic determinant of antibiotic resistance complicates treatment outcomes. The emergence of pathogenic multi-drug resistant *K. pneumoniae* poses a great threat to the healthcare system. Thus, the aim of this study was to isolate and to phenotypically detect the extended-spectrum beta-lactamase produced by *K. pneumoniae* that are isolated from different clinical samples from ICU in Khamis Military Hospital.

Methods: A retrospective observational study was conducted at Khamis Military Hospital from December 2021 to April 2022. Clinical samples collected within the ICU underwent microbiological identification phase to confirm the presence of *K. pneumoniae*. This was followed by antibiotic susceptibility testing to various antimicrobial agents to detect the production of carbapenemases (KPC, NDM-1, OXA-48) and extended spectrum beta lactamase (ESBL). The collected data were subjected to statistical analysis using appropriate tools. Descriptive statistics were used to summarize the demographic and clinical characteristics of the patient population from whom the samples were obtained.

Results: A total of 114 isolates of *K. pneumoniae* were identified from different clinical samples of ICU patients. The percentage of ESBL production constituted 28.94% (33 isolates). Regarding the distribution of carbapenemases production, 43 isolates (37.7%) had the KPC and NDM carbapenemases, while 34 isolates (29.8%) had the OXA-48 carbapenemases.

Conclusions: The high prevalence of *K. pneumoniae* represents a threat to ICU patients, thus, this issue should be treated with urgent attention. Our data informs the need for regular surveillance of antibiotic resistance in pathogenic bacteria in clinical settings for meaningful control of emergence and spreading of multi-drug-resistant *K. pneumoniae*.

Keywords: *Klebsiella*, Multi-drug-resistance, Lactamase, Carbapenemase

INTRODUCTION

K. pneumoniae, a gram-negative bacillus, is a significant contributor to healthcare-associated infections within hospital intensive care units (ICUs). These infections encompass a spectrum including bloodstream infections, urinary tract infections, ventilator-associated pneumonia, and surgical site infections.[1] The pathogenicity of *K. pneumoniae* is attributed to its lipopolysaccharides, cell wall proteins, and envelope receptors, which facilitate its binding to host cells while evading the human immune system.[2] The growing concern arises from the increasing resistance demonstrated by these bacteria, particularly against carbapenem antibiotics, a class previously reserved as the drug of last resort for severe gram-negative bacterial infections.[3,4]

Carbapenem resistance in *Enterobacteriaceae*, including *K. pneumoniae*, is governed by two primary mechanisms. The first entails the production of enzymes such as ESBLs and AmpC beta-lactamases that hydrolyze cephalosporins. The second mechanism involves the production of carbapenemases, a lactamase capable of hydrolyzing a wide range of antibiotics including carbapenems. These carbapenemases include *K. pneumoniae* carbapenemases (KPC), metallo-beta-lactamases such as New Delhi metallo-beta-lactamase-1 (NDM-1), metalloproteinases active on imipenem (abbreviated as IMP), Verona Integron metallo-beta-lactamase (VIM), and oxacillinase-48-like carbapenemases (abbreviated as OXA-48).[5] Notably, KPC demonstrates clonal expansion and genetic exchange capabilities, leading to enhanced resistance development and resilience in human reservoirs through biofilm formation.[6]

The resistance mechanism presented by NDM-1 has a particularly concerning therapeutic implication. First reported in 2009, NDM-1 confers resistance to a vast majority of available antibiotics, leaving only limited options like colistin and tigecycline effective. Moreover, the genes encoding NDM-1 exhibit a propensity for horizontal transfer to other species within the *Enterobacteriaceae* family, exacerbating the challenge of antibiotic resistance dissemination.[7]

Among the ESBLs, three main types are recognized and were named based on their genes: TEM, SHV, and CTX-M. These plasmid-mediated enzymes are associated with genes such as blaTEM, responsible for hydrolyzing penicillins and first-generation cephalosporins, and blaCTX-M, which exhibits a preference for cefotaxime.[8,9] Another ESBL gene, SHV, underpins plasmid-mediated resistance to ampicillin and is frequently identified in enterobacteria like *K. pneumoniae* and *Escherichia coli*.[10]

Given this complex landscape of antimicrobial resistance, this study aimed to isolate and identify ESBLs production in *K. pneumoniae* strains originating from diverse clinical samples within the ICU of Khamis Military Hospital in Abha, Kingdom of Saudi Arabia. Few studies in Saudi Arabia described the pattern of ESPLs production by *K. pneumoniae* in hospitals settings, which highlight the urgent need for conducting this research. This study holds significance in contributing to the understanding of antibiotic resistance patterns in a clinical context, aiding in the formulation of effective therapeutic strategies and measures to counter the escalating challenge of multidrug-resistant bacterial infections.

METHODS

This research was a retrospective observational study conducted at Khamis Military Hospital, Abha, Saudi Arabia. The study aimed to isolate and identify ESBLs produced by *K. pneumoniae* in various clinical samples obtained from patients admitted to the ICU during the period from December 2021 to April 2022. A total of 114 isolates of *K. pneumoniae* were obtained from clinical samples collected within the ICU. These isolates were derived from different types of infections. Clinical samples were collected and stored as per standard procedures to maintain their integrity and prevent contamination.

To include the clinical samples in the study, all samples underwent microbiological identification. During the initial Identification phase, the collected clinical isolates were subjected to initial identification through conventional methods which involved preliminary testing to identify potential *K. pneumoniae* isolates based on their morphological and biochemical characteristics. During the confirmation of isolates phase, the identified isolates were further confirmed using advanced microbiological techniques. This confirmation step aimed to ensure the accuracy of the initial identification and confirm the isolates as *K. pneumoniae* bacteria. Antimicrobial susceptibility testing was then carried out on the confirmed *K. pneumoniae* isolates to assess their response to various antibiotics. This testing was conducted using the Vitek 2 identification system, a product of BioMerieux.[11] The testing was performed in strict adherence to the manufacturer's instructions to ensure consistent and reliable results.

Additionally, data about the clinical samples were collected from patients records, microbiological test results, and antimicrobial susceptibility profiles. Demographic information of the patients from whom the samples were obtained, and sample details were recorded. The collected data were subjected to statistical analysis using appropriate tools. Descriptive statistics were used to summarize the demographic and clinical characteristics of the patient population from whom the samples were obtained. The percentages of ESBL-producing *K. pneumoniae* and carbapenemases were calculated and presented in table. The authors obtained approval from the Scientific Research and Conference Committee of Najran University, Saudi Arabia. Permission for data collection was obtained from Khamis Military Hospital

Ethics Internal Review Board Committee, and the data collected was used for research purposes only.

Following the microbiological identification phase, a total of 114 isolates of *K. pneumoniae* were meticulously collected from diverse clinical samples originating from patients within ICU. Among this collection, the distribution of isolates across different sources was as follows: tracheal secretions contributed to 29 (25.43%) isolates, urine samples contributed to 31 isolates (27.19%), blood specimens contributed to 31 isolates (27.19%), wound swabs contributed to 12 isolates (10.52%), and sputum samples contributed to 11 (9.6%) isolates. In addition, 55 (48.24%) isolates originated from male patients, and 59 (51.75%) originated from female patients (Table 1).

RESULTS

Table 1: *K. pneumonia* isolates collected from different clinical samples of ICU patients in Khamis Military Hospital (n=114).

Type of sample	ESBL	ESBL, KPC, NDM,	KPC, NDM	OXA-48	ESBL, OXA-48	Total
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Urine	19 (57.6)		6 (13)	5 (14.7)	1 (50)	31 (27.2)
Blood	5 (15.2)	1 (5)	18 (41.9)	7 (20.5)		31 (27.2)
Wound swab	4 (12.1)		4 (9)	3 (8.8)	1 (50)	12 (10.5)
Tracheal aspirate	5 (15.1)	1 (5)	12 (27.9)	11 (32)		29 (25.4)
Sputum			3 (7)	8 (23)		11 (9.6)
Total	33 (28.9)	2 (1.7)	43 (37.7)	34 (29.8)	2 (1.7)	114 (100)

Among the isolated *K. pneumoniae* bacteria, the percentage of ESBL production constituted 28.94% (33 isolates). Among these ESBL-producing isolates, 5 (15.15%) were extracted from tracheal secretions, 19 (57.57%) from urine samples, 5 (15.15%) from blood samples, and 4 (12.12%) from wound swabs.

Regarding the distribution of carbapenemases production, among the isolated *K. pneumoniae*, 43 isolates (37.7%) had the KPC and NDM carbapenemases, while 34 isolates (29.8%) had the OXA-48 carbapenemases. The co-occurrence of ESBL mechanism of resistance with the presence of KPC and NDM carbapenemases was found in 2 isolates (1.7%) only, while the co-occurrence of ESBL mechanism of resistance with the presence of OXA-48 carbapenemase was also found in 2 isolates (1.7%) only (Table 1).

DISCUSSION

The emergence of ESBL producing *K. pneumoniae* strains within high-risk settings such as ICUs and their subsequent spread from hospital environments to the community poses a grave challenge to public health. The virulence factors associated with ESBL-producing *K. pneumoniae*, particularly those linked to its capsule, play a pivotal role in its pathogenicity, thereby exacerbating the intricacies involved in infection management. Previous studies have also emphasized the importance of ESBL-producing *K. pneumoniae'*s virulence factors in the complexity of infections management and their potential impact on public health.[12,13] In concurrence with previous findings, this study sheded light on *K. pneumoniae's* significance as a prominent ICU pathogen, capable of rapidly adapting and expanding its resistance spectrum against multiple antimicrobial agents.[14]

The insights gleaned from this investigation stood to heighten physician awareness regarding early diagnosis and targeted antimicrobial interventions to prevent potential complications. The presented data will serve to enhance clinical decision-making, enabling healthcare providers to initiate appropriate treatment strategies promptly.[14] Early detection and responsive therapeutic strategies are critical to mitigate the challenges posed by ESBL-producing *K. pneumoniae* strains.

This study findings underscored the distribution patterns of multi-drug resistant *K. pneumoniae* isolates, revealing a substantial prevalence among various clinical samples. Notably, 27.19% of resistant *K. pneumoniae* were sourced from both urine and blood, with a comparable prevalence of 25.43% in tracheal aspirates, 10.52% in wound swabs, and 9.6% in sputum cultures. This aligned with earlier research that had identified *K. pneumoniae* as a key culprit in urinary tract infections, bloodstream infections, and surgical wound-related infections.[15,16]

Within the confines of Khamis Military Hospital, this study scrutinized 114 isolates of *K. pneumoniae* for their carbapenem resistance profiles. The analysis revealed a spectrum of resistance mechanisms, with ESBL producers constituting 28.94%, OXA-48 carbapenemase production at 29.82%, combined KPC and NDM carbapenemases production at 37.71%, ESBL and OXA-48 carbapenemase co-occurrence at 1.7%, and a similar prevalence of 1.7% for combined ESBL, KPC and NDM resistance. The outcomes of this study were in tandem with reports from North Africa and West Africa, which had also indicated notably elevated phenotypic resistance rates.[3,17]

A precedent for high percentages of ESBL-producing strains and a remarkable incidence of multidrug resistance has been documented previously in Saudi Arabia, specifically within the Armed Forces Hospital in Al-Kharaj, during the period spanning November 2004 to October 2007.[18]

In the current landscape, the escalation of *K. pneumoniae* infections was amplified by the increasing incidence of severe cases, coupled with a dearth of effective therapeutic options. This challenging scenario is compounded by the emergence of *K. pneumoniae* strains that have acquired enhanced virulence or antibiotic resistance traits. The intricacies of these newly evolved factors, their implications in distinct patient cohorts, and the potential targets for intervention remain areas of ongoing research.[19]

CONCLUSION

This study's culmination highlights the prominence of *K. pneumoniae* as a formidable ICU-associated pathogen. Predominantly identified in urine, blood, and respiratory sources, the pervasive presence of multi-drug resistant *K. pneumoniae* necessitates vigilant attention from infection control authorities, particularly within ICU settings. Recommendations stemming from this study advocate for routine in-vitro assays as a mean to expedite tailored treatments, thereby mitigating the potential complications that vulnerable ICU patients face. The study's implications resonate far beyond its immediate scope, emphasizing the urgency for a comprehensive approach to address the evolving challenges of infectious diseases in healthcare settings.

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