Original Article

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Prevalence of Hypervirulent *rmpA* and *magA* Genes in Clinical Isolates of *Klebsiella pneumoniae* and their association with Drug Resistant Pattern: A Cross-sectional Study

Microbiology Section

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ABSTRACT

Introduction: Hypervirulent *Klebsiella pneumoniae* (hvKp) has emerged as a pathogen of global concern that is hypermucoviscous, causing infections and rapidly acquiring Antimicrobial Resistance (AMR). HvKp is more virulent than classical *K. pneumoniae* (cKp) and frequently infects healthy individuals with community-acquired illnesses. Since hvKp commonly exists in the gastrointestinal tract, its communicable spread affects the general public and healthcare facilities. The study was necessary due to the rapid evolution of hvKp, the need to identify novel, reliable agents of hypervirulence and drug resistance, and more reliable targets for therapeutic intervention.

Aim: To investigate the prevalence of hvKp strains (*rmpA* and *magA* genes) and drug-resistant patterns in clinical isolates of *Klebsiella pneumoniae* (*K. pneumoniae*) in a tertiary care hospital in Puducherry, India.

Materials and Methods: The cross-sectional study included a total of 100 non duplicate consecutive isolates of *K. pneumoniae* collected from Mahatma Gandhi Medical College and Research Institute and Hospital, Puducherry, India. These isolates were recovered from various clinical specimens such as urine, sputum, pus, blood, and other miscellaneous specimens mainly obtained from both outpatients and inpatients between August 2021 and April 2022. The antibiotic susceptibility of cultured *K. pneumoniae* was determined by the disk-diffusion test following the 2020 Clinical and Laboratory Standards Institute (CLSI) guidelines. The *rmpA* and *magA* genes were detected using conventional Polymerase Chain Reaction (PCR). PCR products were sequenced using Sanger sequencing. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) statistics software for Windows, version 15.0 (available from SPSS Inc., Chicago, USA). A p-value <0.05 was considered statistically significant.

Results: A total of 100 isolates were studied for phenotypic and genotypic properties. HvKp was identified phenotypically using the string test, and further, the *rmpA* and *magA* genes were identified genotypically using PCR. Out of 100 isolates, the phenotypic study showed 11 (11%) isolates as string test positive. Furthermore, genotypic results showed that 22 (22%) isolates expressed the *rmpA* gene, 11 (11%) isolates expressed the *magA* gene, and 7 (7%) isolates expressed both *rmpA* and *magA*. Most of the hvKp isolates were obtained from urine specimens of *K. pneumoniae* isolates and showed more resistance than cKp towards all classes of antibiotics.

Conclusion: The high prevalence of *rmpA* and *magA* genes suggests their strong role and capability as genetic markers for the identification of hvKp in the laboratory. This study indicated the important role of genetic elements in the emergence of drug resistance in hypervirulent strains and showed that hvKp has a multidrug resistance pattern.

Keywords: Antibiotic drug resistance pattern, Classical *Klebsiella pneumoniae*, Mucoviscosity associated genotype aerobactin gene, Regulator mucoid phenotype aerobactin gene

INTRODUCTION

Hypervirulent *K. pneumoniae* (HvKp) is an invasive variant of *K. pneumoniae* that differs from Classical *Klebsiella pneumoniae* (CKp) with hypermucoviscosity and hypervirulence, causing community-acquired infections, including pyogenic liver abscess, pneumonia, meningitis, and endophthalmitis. HvKp is a major cause of morbidity and mortality in India due to the presence of capsules, siderophores, lipopolysaccharides, fimbriae, Outer Membrane Proteins (OMP), and the type 6 secretion system [1]. Strain-specific capsular polysaccharides, known as K-antigens, shield bacteria from phagocytosis. K1 and K2 are two of the 78 capsular serotypes linked to serious infections in people [2]. Most reported infections due to HvKp have been acquired in the community [1], and the strain infects healthy individuals of any age, with infected patients often presenting with infections at multiple sites. Positive results in the string test have been described as indicators of hypervirulence, and there are limited studies on antibiotic-resistant patterns with HvKp in India [3]. Thus, the *rmpA* gene has been studied as a diagnostic marker for the identification of HvKp.

Strains of *K. pneumoniae* generate a polysaccharide capsule coating as a defense mechanism against the actions of bactericidal agents. On the other hand, a high production of capsule polysaccharides results in an exceptionally thick capsule in certain isolates [4], leading to a hypermucoviscous phenotype. Genes such as Mucoviscosity associated gene A (*magA*), Regulator of mucoid phenotype A (*rmpA*), and Rcs AB have been studied as factors that regulate hypermucoviscosity. Clinical information has also revealed an invasive syndrome and a hypermucoviscous phenotype, with strains having thicker capsules shown to be more virulent [5]. Not all strains of HvKp are hypermucoviscous, and some strains of *K. pneumoniae* also exhibit a hypermucoviscous phenotype.

Additionally, HvKp strains exhibit hypermucoviscosity, a characteristic associated with many hypervirulent isolates. Hypermucoviscosity is a phenotypic description of HvKp in

laboratory settings. The combination of cKp and HvKp AMR determinants on the same or coexisting plasmids has led to the evolution of Multidrug Resistance (MDR) in HvKp [6]. Initially, resistance elements were inserted into the virulence plasmid of HvKp, or resistance plasmids were acquired by HvKp strains, resulting in the acquisition of AMR genes [7]. The acquisition and integration of Integrative Conjugal Elements (ICE) containing AMR determinants into a HvKp strain's virulence plasmid or chromosome constitute the second mechanism [8-10]. The third mechanism involves chromosomal gene disruption mutations (e.g., genes encoding OMP). The fourth mechanism occurs when MDR or Extensively Drug-Resistant (XDR) cKp strains obtain the HvKp virulence plasmid [11,12].

The aim of this study was to investigate the prevalence of HvKp strains (*rmpA* and *magA* genes) and drug-resistant patterns in clinical isolates of *K. pneumoniae* in a tertiary care hospital in Puducherry, India.

The primary objectives were to first identify the HvKp strains using the string test and analyse the drug-resistant patterns for HvKp isolates in phenotypic characterisation. Furthermore, the HvKp genes *rmpA* and *magA* were identified using PCR in genotypic characterisation. The secondary objective was to conduct a comparison of HvKp and cKp in phenotypic and genotypic analysis, along with their antibiotic resistance patterns in clinical isolates of *K. pneumoniae.*

MATERIALS AND METHODS

This cross-sectional study was conducted at the Mahatma Gandhi Medical College and Research Institute (MGMCRI) of Sri Balaji Vidyapeeth University in Puducherry, India, from August 2021 to April 2022. The study was approved by the Institutional Human Ethics Committee (IHEC) of the MGMCRI of Sri Balaji Vidyapeeth University, Puducherry, India (MGMCRI/RAC/02/2020/XX/IHEC/137).

This study includes *K. pneumoniae* isolates obtained from various clinical samples such as blood, urine, pus, tissue, body fluids, swabs, and respiratory specimens from both inpatients and outpatients. The samples were collected during the specified study duration as this was a time-bound study.

Inclusion criteria: All the clinical isolates of *K. pneumoniae* were included in the study.

Exclusion criteria: Repeated isolates of *K. pneumoniae* from the same patients were excluded from the study.

Isolates collection and processing: One hundred clinical isolates of *K. pneumoniae* were collected from both inpatients and outpatients. HvKp isolates were identified phenotypically using the string test [13], and the *rmpA* and *magA* genes were further detected genotypically using conventional PCR. Sequencing was performed using Sanger sequencing [14].

Antibiotic susceptibility test: Antibiotic susceptibility tests were performed using the Kirby-Bauer disc diffusion method following CLSI guidelines for 2020 [15]. All *K. pneumoniae* isolates were tested for their resistance against the following antibiotics: Cotrimoxazole (10 µg), ceftriaxone/cefotaxime (10/30 µg), ciprofloxacin/norfloxacin (5/10 µg), gentamicin (10 µg), amikacin (30 µg), imipenem (10 µg), meropenem (10 µg), piperacillin + tazobactam (100/10 µg), cefoperazone + sulbactam (100/10 µg), and fosfomycin (200 µg).

The antibiotic susceptibility of cultured *K. pneumoniae* was determined using the disk diffusion test. After uniformly plating the cultivated cells on Mueller Hinton agar, they were allowed to dry for five minutes. Antibiotic disks (HiMedia) were then placed on the agar surface. After 24 hours of incubation at 37°C, the diameters of the inhibition zones were measured using a metric ruler.

Phenotypic test for Hypervirulent *K. pneumoniae*: Observing colony morphology, haemolytic characteristics, gram staining, capsule staining, pigment production (in blood and MacConkey agar media, and CLED agar for urine samples), as well as biochemical tests (including the oxidase test, catalase test, and other biochemical reactions after inoculation in triple sugar iron agar, Mannitol motility medium, Indole, Urease, and Simmons' citrate agar media) were used to identify the organism phenotypically. Additionally, some biochemical reactions (lysine, ornithine) were utilised to detect subspecies of the organism. Isolated *K. pneumoniae* were stored at -22°C for future use after being subcultured in MacConkey agar media and stock cultured in semisolid nutrient agar and glycerol.

Colonies grown on agar plates exhibited a pathognomonic or hypermucoviscous appearance [15]. If, when stretching bacterial colonies on an agar plate using a bacteriology inoculation loop or needle, a viscous string of >5 mm in length was obtained, the test was considered positive [15]. The length of the viscous string was measured by placing a ruler directly behind it [16].

Polymerase Chain Reaction (PCR): The DNA extraction of the preserved samples was conducted using a commercially available DNA extraction kit (HiMedia, India), and the target genes (*rmpA*, *magA*) were amplified using appropriate primers (Sigma-Aldrich) and master mix (Amplicon) in a thermal cycler (CFX 96, Bio-Rad, California, USA).

The primers used for *magA* were:

magA-F: 5'GGTGCTCTTTACATCATTGC-3'

magA-R: 5'GCAATGGCATTTGCGTTAG-3' [17]

The primers used for *rmpA* were:

rmpA-F: 5'ACTGGGCTACCTCTGCTTCA-3'

rmpA-R: 5'CTTGCATGAGCCATCTTTCA-3' [17]

The reaction was carried out with an initial activation at 95°C for 30 seconds, followed by annealing at 60°C for 90 seconds, extension at 72°C for 60 seconds, and a final extension at 72°C for 10 minutes. The amplified products were loaded onto 2% agarose gels stained with ethidium bromide and visualised under ultraviolet light using the Geldoc XR system (Bio-Rad, California, USA). The size of the amplicon product for the *rmpA* gene is 516 basepairs, and for the *magA* gene is 1283 basepairs. A 100 basepair DNA ladder was used for this study.

DNA Sequencing: PCR-positive amplicons were purified and sequenced using the BigDye 3.1 Cycle Sequencing Kit with Sanger sequencing (Eurofins, India). The nucleotide sequences were analysed and compared with the sequences available at the National Center for Biotechnology Information, which were also used in a similar article in the literature [14].

STATISTICAL ANALYSIS

The data collected from the *K. pneumoniae* isolates and their phenotypic and genotypic analyses were further studied and compared using the Chi-square test and Fisher's exact test through statistical analyses conducted using SPSS, a statistical software for Windows, version 15.0 (available from SPSS Inc., Chicago, IL, USA). A p-value of < 0.05 was considered statistically significant.

RESULTS

The distribution of samples and types of patients with *K. pneumoniae* isolates was examined. The results showed that the majority of infections occurred in the 61-70 age group, with 27 (27%) cases, followed by the 51-60 age group with 21 (21%) patients. The age range of patients included in the study was 1-89, with a mean age of 58.16±17.27 years. Among the distribution, urine samples had the highest number of *K. pneumoniae* isolates at 46 (46%), followed by pus swab samples at 26 (26%). In terms of patient type, 71 (71%) of the *K. pneumoniae* isolates were collected from inpatients, while 29 (29%) were from outpatients. Male patients had a higher infection rate at 64 (64%) compared to female patients at 36 (36%) [Table/Fig-1].

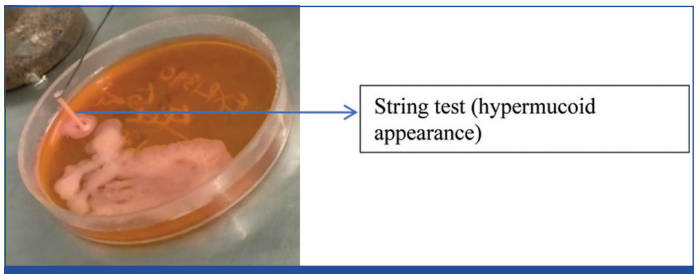
Out of the 100 isolates, 11 (11%) tested positive in the string test for phenotypic identification. Among these 11 isolates, seven were positive for the *rmpA* gene, four were positive for the *magA* gene, and three were positive for both *rmpA* and *magA*. The remaining 89 isolates tested negative in the string test. Out of these 89 isolates, 15 were positive for the *rmpA* gene, seven were positive for the *magA* gene, and four were positive for both *rmpA* and *magA* genes.

The majority of hypermucoviscous *K. pneumoniae* (hvKp) isolates were obtained from urine specimens [Table/Fig-1]. In the present study, it was observed that inpatients exhibited a higher number of string-positive isolates phenotypically compared to outpatients. This could be attributed to their prolonged hospital stays, exposure to invasive procedures necessary for their treatment, extended use of invasive devices, and multiple antimicrobial therapies, making inpatients more susceptible to nosocomial infections than outpatients.

Sample	String test negative, n (%)	String test positive, n (%)	Male, n (%)	Female, n (%)	Inpatient, n (%)	Outpatient, n (%)	Total, n (%)
Broncho Alveolar Lavage	1 (1.12)	0	1 (1.56)	0	1 (1.40)	0	1 (1)
Blood	2 (2.24)	0	2 (3.125)	0	2 (2.81)	0	2 (2)
Entotracheal aspirate	3 (3.37)	1 (9.0)	4 (6.25)	0	2 (2.81)	2 (6.89)	4 (4)
Liver aspirate	1 (1.12)	0	1 (1.56)	0	1 (1.40)	0	1 (1)
Pleural fluid	1 (1.12)	0	1 (1.56)	0	1 (1.40)	0	1 (1)
Pus swab	23 (25.8)	3 (27.2)	17 (26.56)	9 (25)	18 (25.35)	8 (27.5)	26 (26)
Sputum	8 (8.9)	1 (9.0)	4 (6.25)	5 (13.8)	7 (9.85)	2 (6.89)	9 (9)
Tissue	3 (3.37)	1 (9.0)	4 (6.25)	0	2 (2.81)	2 (6.89)	4 (4)
Urine	41 (46.0)	5 (45.4)	29 (45.3)	17 (47.2)	31 (43.66)	15 (51.72)	46 (46)
Vaginal swab	3 (3.37)	0	0	3 (8.33)	3 (4.22)	0	3 (3)
Wound swab	3 (3.37)	0	1 (1.56)	2 (5.55)	3 (4.22)		3 (3)
Total	89	11	64	36	71	29	100

[Table/Fig-1]: Sample-wise distribution in string test, gender and type of patients.

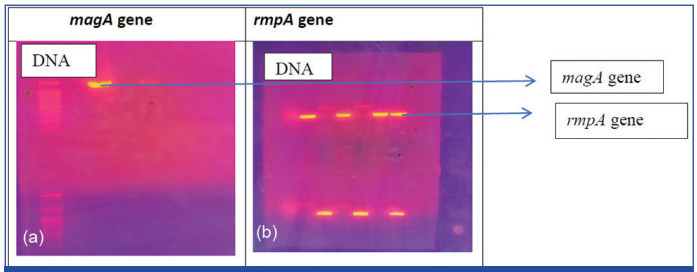
The string test, a simple laboratory procedure, may aid in the early diagnosis of hvKp infections. On agar plates, hvKp is characterised by a hypermucoid appearance [Table/Fig-2].



[Table/Fig-2]: String test (Hyper mucoid appearance).

PCR amplification of the *magA* gene among *K. pneumoniae* isolates: Lane 1 shows a 100 bp DNA ladder, Lanes 2, 4, and 6 were negative, while Lane 3 shows a positive result. The *magA* gene amplification resulted in a 1283 basepair product [Table/Fig-3a].

PCR amplification of the *rmpA* gene among *K. pneumoniae* isolates: Lane 1 displays a 100 bp DNA ladder, Lanes 2, 4, 6, and 7 show positive results, while Lanes 3 and 5 show negative results. The *rmpA* gene amplification resulted in a 516 basepair product [Table/Fig-3b].



[Table/Fig-3]: Genotypic and phenotypic characterisations. (a) Lane1 DNA ladder, lane 2,4,5,6 were negative. Lane 3 were positive for *magA* gene-1283 basepair; (b) Lane1 DNA ladder, lane 2,4,6,7 were positive for *rmpA* gene and lane 3,5 were negative.

The antibiotic resistance pattern for the clinical isolates of cKp and hvKp is presented in [Table/Fig-4]. HvKp exhibited higher antibiotic resistance rates compared to cKp across all classes of antibiotics, such as cotrimoxazole with 16 isolates (72.70%), ceftriaxone/cefotaxime with 22 isolates (100%), ciprofloxacin/norfloxacin with 18 isolates (81.80%), gentamicin with 17 isolates (77.30%), amikacin with 10 isolates (45.5%), imipenem with 11 isolates (50%), meropenem with 14 isolates (63.6%), piperacillin + tazobactam with eight isolates (36.40%), cefoperazone + sulbactam with nine isolates (40.90%), and fosfomycin with five isolates (22.70%) [Table/Fig-4].

Antibiotics	ckp (78), n (%)	hvKp (22), n (%)
Cotrimoxazole (10 µg)	19 (24.4)	16 (72.7)
Ceftriaxone/Cefotaxime (10 µg)	54 (69.2)	22 (100)
Ciprofloxacin/Norfloxacin (5/10 µg)	33 (42.3)	18 (81.8)
Gentamicin (10 µg)	22 (28.2)	17 (77.3)
Amikacin (30 µg)	16 (20.5)	10 (45.5)
Imipenem (10 µg)	12 (15.4)	11 (50)
Meropenem (10 µg)	19 (24.4)	14 (63.6)
Piperacillin+Tazobactam (100+ 10 µg)	2 (2.6)	8 (36.4)
Cefoperazone +Sulbactam (100+ 10 µg)	11 (14.1)	9 (40.9)
Fosfomycin (200 µg)	1 (1.3)	5 (22.7)

[Table/Fig-4]: Antibiotic resistant pattern of ckp and hvKp.

According to [Table/Fig-5], there was a higher antibiotic resistance pattern among inpatients than outpatients. Antibiotic resistance shows a statistically significant difference between inpatients and outpatients only in the case of Amikacin (p-value=0.023).

Antibiotics	Inpatient, n (%)	Outpatient, n (%)	Chi-square value	p-value
Cotrimoxazole (10 µg)	27 (38.02)	8 (27.58)	0.987	0.321
Ceftriaxone/Cefotaxime (10 µg)	56 (78.87)	20 (68.96)	1.108	0.292
Ciprofloxacin/Norfloxacin (5/10 µg)	38 (53.5)	13 (44.8)	0.623	0.43
Gentamicin (10 µg)	31 (43.66)	8 (27.58)	2.237	0.135
Amikacin (30 µg)	23 (32.39)	3 (10.34)	5.203	0.023##
Imipenem (10 µg)	17 (23.94)	6 (20.68)	0.123	0.726
Meropenem (10 µg)	23 (32.39)	10 (34.48)	0.041	0.84
Piperacillin+Tazobactam (100+10 µg)	6 (8.45)	4 (13.79)	0.653	0.47
Cefperazone+Sulbactam (100+10 µg)	16 (22.53)	4 (13.79)	0.983	0.321
Fosfomycin (200 µg)	4 (5.63)	2 (6.89)	0.058	0.99

[Table/Fig-5]: Antibiotic resistant pattern with inpatient and outpatient.
##Fischer-exact test applied

In [Table/Fig-6], string test-positive, *rmpA*-positive, and *magA*-positive isolates expressed greater in inpatients compared with outpatients. Both *rmpA* and *magA* genes were expressed more in outpatients compared with inpatients.

Isolates (N=100), n (%)	Positive Isolates, n (%)	Inpatient, n (%)	Outpatient, n (%)	Pearson Chi-square value	p-value
String test positive	11 (11)	8 (11.3)	3 (10.3)	0.018	0.99
rmpA gene positive	22 (22)	16 (23.9)	6 (20.6)	0.041	0.84
magA gene Positive	11 (11)	6 (8.5)	5 (17.2)	1.625	0.289
Both *rmpA* and *magA* gene positive	7 (7)	3 (4.2)	4 (13.8)	2.895	0.189

[Table/Fig-6]: Association of type of patients with string test, *rmpA*, *magA*, both *rmpA* and *magA* gene isolates.

DISCUSSION

In recent years, *K. pneumoniae* has been characterised as an opportunistic pathogen connected to the healthcare setting, capable of producing life-threatening infections, and now considered a repository of AMR and hypervirulence genes [18]. Present research focused on the prevalence of hvKp in the collected clinical isolates that have certain hypervirulence characteristics and antimicrobial resistant patterns compared to cKp.

In terms of gender distribution, present study found that male patients were predominantly affected by *K. pneumoniae* compared to females. Male patients may have a slight edge over females in terms of infection risk. Siu LK et al., examined several investigations on hepatic abscesses caused by hvKp and identified geographical disparities, with males being more likely to be infected than females overall (55%-483/871) [19]. In the United States, South Korea, and Taiwan, males accounted for infections in 68% (26/38), 42% (136/321), and 63% (321/512) of cases, respectively [20]. Additionally, 80.3% (49/61) of the patients with hvKp infection complicated by endophthalmitis were men [21]. On the other hand, in another investigation, hvKp strains causing liver abscesses affected both men and women. Males comprised 52.5% (21/40) while females accounted for 47.5% (19/40) [20]. In present study, inpatients were found to be infected by *K. pneumoniae* in urine samples compared to outpatients.

In the present study, phenotypic results show that 11% were identified as string test positive. Out of 100 isolates, 22% were found to have the *rmpA* gene and 11% had the *magA* gene. In another study from Bangladesh, the virulence genes *magA* and *rmpA* were detected in 42.86% and 52.38% of isolated string test-positive strains [22]. Studies from China and Egypt reported the presence of these genes in 44.4% and 56.52% of isolated *K. pneumoniae*, respectively [23,24]. In Yu WL et al., showed that the prevalence of HV, *rmpA*, and *magA* were 38%, 48%, and 17%, respectively [17]. A study in India reported 31.3% string-positive strains [25], while another study in Egypt reported 40.71% string test-positive *K. pneumoniae*. The dissimilarities may be due to time, source of sample collection, and geographical variation.

In a study by Raj S et al., from India, out of 120 *K. pneumoniae isolates*, 9.16% tested positive for the string test, and 14 were positive for iucA using PCR [26]. Fang CT et al., reported a higher prevalence of the hypermucoviscous phenotype in invasive strains of liver abscess compared to non invasive strains without liver abscess (98% vs. 17%) [27]. Remya P et al., from southern India reported that very few (5.1%) carried the *rmpA* gene, which co-existed with *magA* in 5% (1/20), K2A in 10% (2/20), and non-*magA*/K2A in 85% (17/20) [28]. Additionally, a substantial number of study isolates carried the *rmpA* gene (62.5%). Al-Jailawi MH et al., also observed the presence of the *rmpA* gene in K1, K2, and non-K1/K2 serotypes in 21.7%, 45.55%, and 16.7% of *K. pneumoniae*, respectively [29]. In Taiwan, all *rmpA*-positive isolates coharbored *magA* and K2A [30].

Zamani A et al., showed that out of 105 *Klebsiella* isolates from patients, 96.2% were *K. pneumoniae*. They detected the *magA* gene in 4 (3.8%) isolates of *K. pneumoniae*, with two of them being positive and two being negative for the HV phenotype [31]. The definition of hvKp is still up for debate despite ongoing studies by various authors globally. Recent research has shown that hypermucoviscosity and hypervirulence are two distinct traits, typically described by hypermucoviscosity with a positive string test. Apparently, hypermucoviscosity is not necessary for the hypervirulent phenotype of *K. pneumoniae*.

In the study by Vandhana V et al., the majority of the isolates were obtained from cases of Urinary Tract Infection (UTI) (34.88%) [13]. In present study, hvKp strains showed resistance to all classes of antibiotics, such as cotrimoxazole 16/22 (72.70%), ceftriaxone/cefotaxime 22/22 (100.0%), ciprofloxacin/norfloxacin 18/22 (81.80%), gentamicin 17/22 (77.30%), amikacin 10/22 (45.50%), imipenem 11/22 (50.00%), meropenem 14/22 (63.60%), piperacillin + tazobactam 8/22 (36.40%), cefoperazone + sulbactam 9/22 (40.90%), and fosfomycin 5/22 (22.7%).

The present study showed that more hvKp (22%) were detected in urine samples. Related to the present study, in Australia from 2001-2014, the source of hvKp in urine, out of 193 isolates, three were identified [32]. This may be due to faecal flora causing the majority of UTIs by climbing through the urethra to infect the bladder and occasionally reaching the kidneys and systemic circulation. Infection is brought on by Ckp in this way. Although biologically feasible, this pathogenic mechanism for hvKp is less well established. It is claimed that the urinary tract is a source of hvKp bacteraemia, but the main mechanism by which hvKp establishes bacteraemia infection appears to be independent of the urinary tract. On the contrary, hematogenous seeding from earlier bacteraemia causes the majority of genito-UTIs identified as being caused by hvKp [32].

In an Indian study, between 2014 and 2015, out of 370 isolates, 3 (0.81%) isolates were identified as hvKp in the source of urine, respiratory, and blood isolates [14]. Another study showed that out of 191 isolates, 1 (0.052%) isolate was identified as hvKp in the source of the urine sample [20]. Similarly to cKp, hvKp strains are becoming more resistant to antimicrobials through the acquisition of mobile elements containing resistance determinants. Additionally, new hvKp strains arise when cKp strains that are XDR acquire virulence determinants specific to hvKp, leading to nosocomial infection. No controlled trials have evaluated the effectiveness of different antimicrobials against infections caused by hvKp. This is partly because clinical microbiology labs are unable to distinguish between strains of cKp and hvKp. Subsequent clinical trials should be made possible by the recent discovery of biomarkers that reliably identify hvKp strains. For hvKp to be effectively controlled in the clinical setting, efficient diagnostic tools for hvKp identification must be developed. It is also imperative that new antibacterial drugs be developed.

Limitation(s)

This study has certain limitations, such as being a single-centre study, and the clinical infection may vary in different geographical regions.

CONCLUSION(S)

The high prevalence of *rmpA* and *magA* genes suggests their strong capability for rapid and accurate diagnosis, as well as their role as genetic markers for the identification of hvKp in the laboratory. This study indicates the important role of genetic elements in the emergence of drug resistance in hypervirulent strains and has revealed the existence of hvKp strains and their increased tendency towards drug resistance. Additionally, hvKp showed a MDR pattern

towards all classes of antibiotics. It is important to identify these genetic determinants behind the hypervirulent phenotype in order to develop an effective treatment protocol.

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