JOURNAL OF CLINICAL IMMUNOLOGY AND MICROBIOLOGY

E-ISSN: 3068-5168
Journal of Clinical Immunology & Microbiology

ATHENAEUM SCIENTIFIC PUBLISHERS

Research Article

Comparative Analysis of Environmental Versus Clinical Isolates of *Klebsiella pneumoniae*: A Key Trafficker of Antibiotic Resistance Genes (AMR)

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Citation: Singh AN, et al. Comparative Analysis of Environmental Versus Clinical Isolates of *Klebsiella Pneumoniae*: A Key Trafficker of Antibiotic Resistance Genes (AMR). J Clin Immunol Microbiol. 2025;6(2):1-12.

http://dx.doi.org/10.46889/JCIM.2025.6208

Received Date: 03-07-2025

Accepted Date: 21-07-2025

Published Date: 28-07-2025

Abstract

Antibiotic resistance, along with hypermucoviscosity and carbapenem-colistin *Klebsiella pneumoniae*, is upsurging at an alarming rate. It has been suggested that additional drug resistance can sometimes emerge due to mutations in chromosomal genes and the majority of AMR genes in *K. pneumoniae* stem from acquiring large and small plasmid consignments through horizontal gene transfer. The selection pressure of antibiotics in the clinical environment results in the accumulation of up to 400 AMR genes, leading to the emergence of Pan-Resistant Strains (PDR).

In this study, a total of 129 clinical isolates (Pus, blood, urine, CSF, sputum, tissue and stool) and 120 environmental isolates (University hospital sewage, sewage, soil, ponds and different sites (ghats) of the river Ganga) were analysed. The collected strains (clinical and environmental) were identified using *K. pneumoniae* species-specific primers targeting the 16S-23S rDNA Internal Transcribed Spacer (ITS) region, followed by the molecular charcaterization by Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR. The antimicrobial resistance pattern of clinical and environmental isolates was assessed by disk diffusion (Kirby-Bauer method) or broth microdilution (colistin).

The antibiotic resistance pattern of clinical-origin *K. pneumoniae* was observed to be the most resistant against all the antibiotics, except imipenem (32.6%) and colistin (15.5%). The environmental origin *K. pneumoniae* exhibits a lower resistance pattern against all antibiotics, with meropenem and imipenem being the most effective antibiotics, having 6.7% and 8.4% resistance rates, respectively.

The high selection pressure of antibiotics in the hospital environment leads to the accumulation of a variety of resistance genes in plasmid-borne, sturdy *K. pneumoniae*, resulting in the incessant exchange of Antibiotic Resistance (AMR) genes between the natural environment and the hospital setup.

Keywords: *Klebsiella pneumoniae*; Antibiotic Resistance (AMR); Pan-Resistant Strains (PDR)

Introduction

Antibiotic Resistance (AMR) in *Klebsiella pneumoniae* is emerging as a serious crisis worldwide. Moreover, *K. pneumonaie* is a significant member of the ESKAPE group of microorganisms responsible for causing various community-acquired and nosocomial infections such as septicemia, pneumonia, urinary tract infection, liver abscesses, meningitis and surgical site infection, with an increased mortality rate in immunocompromised individuals, neonates and the elderly [1-3]. Horizontal Gene Transfer (HGT) results in the distribution and accumulation of more than a hundred mobile AMR genes in the plasmid and Mobile Genetic Elements (MGEs), including transposons and integrons, posing an urgent threat to human health [4-8].

Recently, the Global Antimicrobial Resistance and Use Surveillance System (GLASS): Early Implementation 2020 reported a significant increase in the antimicrobial resistance pattern of *K. pneumoniae* in many countries, leading to life-threatening infections in community and healthcare settings [9]. Moreover, the antimicrobial resistance pattern of *K. pneumoniae* against third-generation cephalosporin was 57.6% in bloodstream infections. Moreover, 12 countries reported 80% to 100% resistance. Globally, the resistance rate of *K. pneumoniae* against third-generation cephalosporin was 80% to 100% and the median resistance rate was 17% against carbapenem [10].

A recent European survey of carbapenemase-producing *Enterobacteriaceae* (EuSCAPE) in 2017 said that carbapenemase resistance genes encoded by the residing plasmid were responsible for causing hospital-acquired infections. Among them, CTX-M-15 causes 45% of disease, oxacillinase-48 (OXA-48-like) causes 37%, New Delhi Metallo-beta-lactamase (NDM) causes 11% and Verona Integron-encoded Metallo-beta-lactamase (VIM) causes 8% infections respectively [11,12]. Colistin remains the last resort antibiotic against carbapenem resistance *K. pneumoniae* worldwide; however, frequent over/misuse of colistin results in colistin resistance in the health care sector. The current scenario of colistin resistance *K. pneumoniae* is upsurging at an alarming rate; before 2015, the colistin resistance was 2.9%; similarly, from 2016-2019, it was 2.9%, respectively. However, the current data on colistin resistance from bloodstream infections after 2020 was 12.9%. Additionally, the highest colistin resistance of 19.2% was reported from Thailand [13].

Therefore, the present study was planned to carry out the isolation and characterization of *K. pneumoniae* from clinical and environmental sources and the determination of antibiotic drug-resistant pattern and clonal relationship of *K. pneumoniae* strains by ERIC PCR and phage typing in a tertiary level hospital and at different sites of North India respectively.

Materials and Methods

Bacterial Strains

One hundred twenty-nine clinical isolates (blood, pus, CSF, sputum, tissue, urine and stool) of *K. pneumoniae* were collected from the patients admitted to Sir Sundarlal Hospital, Varanasi, India over the period of eighteen months from February 2020 to August 2021. Furthermore, one hundred twenty environmental isolates were collected from sewage water, university hospital sewage, ponds, soil and different sites (ghats) of river Ganga in Varanasi. The collected strains (clinical and environmental) were identified using *K. pneumoniae* species-specific primers16S-23S rDNA Internal Transcribed Spacer (ITS) region [14]. The Ethical Committee of Banaras Hindu University, Varanasi, India, approved the study protocol.

Determination of Antimicrobial Resistance

Determination of antimicrobial resistance pattern of clinical and environmental isolates was assessed by disk diffusion (Kirby-Bauer method) or broth microdilution (colistin) methods following the Clinical and Laboratory Standards Institute guidelines (CLSI, 2020). The antimicrobial agents tested by disk diffusion method (Kirby-Bauer method) were ampicillin (10 µg), amoxicillin + clavulanic acid (20/10µg), cefazolin/cephalexin (30µg), cefuroxime (30µg), cefotaxime/ceftriaxone (30µg), gentamicin (10µg), amikacin (30µg), trimethoprim + sulfamethoxazole (1.25µg + 23.75µg), levofloxacin (5µg), piperacillin + tazobactam (100µg + 10µg), cefpirome/cefepime (30µg), imipenem (10µg), ertapenem (10µg), meropenem (10µg). *K. pneumoniae* ATCC 700603 was used as reference strain in the study.

The clinical and environmental strains were classified according to their Antimicrobial Resistance Profile (AMR) as Susceptible (S) and Resistance (R). The clinical and environmental strains were further categories into Multidrug-Resistant (MDR), Extensively Drug-Resistant (XDR) or Pandrug-Resistant (PDR) based on the AMR profile [15].

String Test

The string test was used to confirmed the Hypermucoviscous (HMV) phenotype of *K. pneumoniae*. The bacterial strains were grown in Luria-Bertani (LB) agar at 37°C overnight. The bacteriological inoculation loop was used to generate the viscous string >5 mm by stretching mucous bacterial colonies on LB plate.

Molecular Characterization of Clinical and Environmental Isolates Using 16S-23S rDNA Internal Transcribed Spacer (ITS) Region PCR Product

The clinical and environmental *K. pneumoniae* isolates were molecularly characterized with *K. pneumoniae* species specific 16S-23S rDNA ITS region primer described earlier [8,14,16]. The amplification of 16S-23S rDNA ITS region primer PCR were performed in T100 Thermal Cycler, BIO-RAD, CA, USA. Moreover, the 16S-23S rDNA ITS PCR product was resolved in the 1.5% agarose. A molecular size marker of 1 Kb and 100 bp DNA ladder (GeNeiTM Banglore, India) was used as a molecular marker. The electrophoresed gel was imagined using a gel documentation system (BioRad, Universal Hood II, United States).

Molecular Characterization of Clinical and Environmental Isolates of K. pneumoniae with Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR

The *K. pneumoniae* isolates were molecularly characterized with ERIC PCR using the ERIC forward primer (5′-ATGTAAGCTCCTGGGGATTCAC-3′) and ERIC reverse primer (5′-AAGTAAGTGACTGGGGTGAGCG-3′) [17]. The amplification of ERIC PCR in a thermal cycler was performed in T100 Thermal Cycler, BIO-RAD, CA, USA. Moreover, the ERIC PCR product was resolved in the 1.5% agarose. A molecular size marker of 1 Kb and 100 bp DNA ladder (GeNeiTM Banglore, India) was used as a molecular marker. The electrophoresed gel was imagined using a gel documentation system (BioRad, Universal Hood II, United States).

Dendrogram Based on ERIC PCR Electrophoresis

The Excel sheet of ERIC PCR product of both clinical and environmental isolates banding pattern with the presence of ERIC band represented by 1 and the absence of ERIC band represented by 0. The Excel sheet was used as input data for generating cluster analysis of ERIC segments in the bacterial genome with the NTSYS pc2.0 program of UPGMA software [18].

Antibiotic Drug Susceptibility Testing

Antibiotic drug susceptibility testing was performed by the disk diffusion method described by the Kirby-Bauer disc diffusion method. The standard bacterial inoculum of 0.5 OD (0.5 MacFarland) ≃ 1.5 × 108 CFU/mL was swabbed on the Muller Hinton Agar (MHA) with sterile cotton swab stick followed with standard antibiotic disc procured from HI media, Mumbai, India. *K. pneumoniae* ATCC 700603 as a reference strain was used in the study. The zone of inhibition was measured and calculated according to the procedures of the Clinical and Laboratory Standards Institute [19,20].

Dendrogram Based on Antibiotic Drug Susceptibility Pattern

The Excel sheet of antibiotic drug susceptibility patterns of clinical and environmental isolates was used as input data for generating cluster analysis of antibiotic drug-resistant patterns using the NTSYS pc2.0 program of UPGMA software [18]. An Excel sheet of antibiotics drug susceptibility patterns where the sensitive was represented by 1 while resistant was represented by 0.

Results

Molecular Identification of K. pneumoniae

The amplified PCR product with 16S-23S rDNA ITS region primer gave a single band of 130 bp, confirming *K. pneumoniae* (species level). Thus, 129 isolates of clinical samples were found to be *K. pneumoniae*, whereas 120 isolates environmental isolates were *K. pneumoniae*.

Antibiotic Susceptibility Testing of Clinical Isolates by Disk Diffusion Method

When compared to the environmental isolates, the *K. pneumoniae* of clinical origins were observed to be most resistant against nearly all the antibiotics drugs except imipenem and polymyxin-B. The antibiotic drug resistance pattern of clinical origin against ampicillin, amoxicillin + clavulanic acid, cefazolin/cephalexin and cefuroxime was 100%, followed by cefotaxime/ceftriaxone (98.4%), gentamicin (96.9%), amikacin (91.4%), ciprofloxacin (99.2%), trimethoprim + sulfamethoxazole (96.9%), levofloxacin (99.2%), piperacillin + tazobactam (87.6%), cefpirome/cefepime (96.1%), ertapenem (86.8%), meropenem (72.1%) and imipenem (32.6%). Colistin was the most effective antibiotic, with a resistance rate of 15.5% (Table 1,Fig. 1,2).

The dendrogram was constructed based on the resistance pattern of 129 clinical isolates of *K. pneumoniae*. It showed five major clusters, cluster I, II, III, IV and V. Cluster I contribute the major portion of the dendrogram and has 4 subclusters with identical

pairs and 3 non-similar isolates, KpnBHU35, KpnBHU36 and KpnBHU82. Cluster II contained two isolates, KpnBHU46 and KpnBHU103; Cluster III consisted of 2 subclusters with identical pairs (127) and was identical based on the antibiotic sensitivity pattern; Cluster IV had only two isolates, KpnBHU19 and KpnBHU89 Furthermore Cluster V contains 2 identical pairs (Fig. 3) and antimicrobial resistant of clinical isolates of *K. pneumoniae* based on the dendrogram pattern (clusters).

When the dendrogram was further analyzed based on the identical resistance pattern, it was found that 19 of 129 clinical isolates (14.7%) were resistant to all 5 classes of antibiotics used in antibiotics sensitivity testing. There were 107 of 129 isolates (82.9%) sensitive only to the colistin group of antibiotics. Only 3 strains of 129 isolates (2.3%) were resistant to 3 classes of antibiotics and sensitive to colistin and Trimethoprim + Sulfamethoxazole combination (Table 5).

When we further analyzed the data, we found that 51/129, (39.5%) of the isolates were resistant to 4 groups of antibiotics used with an identical spectrum of sensitivity. There were 16 antibiotics belonging to 5 different classes used in the sensitivity testing and these 39.5% strains were sensitive to imipenem and colistin.

Antibiotic Susceptibility Testing of Environmental Isolates of K. pneumoniae

In antibiotics sensitivity testing of 120 environmental isolates of *K. pneumoniae* with 15 antibiotics, the highest resistance pattern was observed with cefazolin/cephalexin (98.3%), cefuroxime (90.8%) followed by piperacillin + tazobactam (71.4%), cefpirome/cefepime (62.2%), ampicillin (62.2%), cefotaxime/ceftriaxone (59.7%), ertapenem (52.9%), amoxicillin + clavulanic acid (48.7%), amikacin (47.1%). However, other antibiotics, such as gentamicin, ciprofloxacin, trimethoprim + sulfamethoxazole and levofloxacin, have less than a 40% resistance rate. Meropenem and imipenem was the most effective antibiotic, with 6.7% and 8.4% resistance rates against the environmental isolates (Table 1 and Fig. 4,5).

The dendrogram was constructed based on the resistance pattern of 120 environmental isolates of *K. pneumoniae*, showing six major clusters: I, II, III, IV, V and VI. Cluster I contained 8 subclusters with identical pairs and cluster II contained 5 isolates with no identical pairs. Cluster III contained 4 isolates, cluster IV contained 13 isolates with 2 identical pairs, Cluster V consisted of only two different EKpBHU119 and EKpnBHU33 isolates and Cluster VI consisted of 14 isolates with 1 identical pair based on the antibiotics sensitivity pattern (Fig. 6) and antimicrobial resistance of environmental isolates of *K. pneumoniae* based on the dendrogram pattern (clusters).

The antimicrobial resistance pattern of 120 environmental *K. pneumoniae* isolates. Interestingly, none of the environmental isolates was PDR and only 10.8% (13/120) isolates were resistant to 3 or more classes of antibiotics, which may be designated as MDR. There were, 21.6% (26/120) of *K. pneumoniae* isolates were resistant to 1 or other antibiotics belonging to only 2 classes and the majority of isolates, 66.7% of the environmental *K. pneumoniae*, resistant to only the β-lactam group of antibiotics. Further, it is interesting to observe that only 5% (6/120) of environmental isolates had an identical spectrum of antibiotic sensitivity.

Genotyping of Clinical and Environmental K. pneumoniae Isolates by ERIC PCR

Whole genome analysis of all the randomly selected 70 clinical and 73 environmental isolates of *K. pneumoniae* based on ERIC PCR product. UPGMA analysis of 30 randomly selected *K. pneumoniae* isolates from clinical and 30 randomly selected isolates from environmental origin samples were analyzed. Apparently, there were 8 well-defined clusters, clusters I, II, III, IV, V, VI, VII and VIII, where branching occurred at the similarity level of 25%. The cluster-I had 12 strains comprising 7 clinical and 6 environmental origins with two identical pairs (KpnBHU30 and EKpnBHU14; KpnBHU54 EKpnBHU24 and EKpnBHU56). Cluster II contributes a significant portion of the dendrogram comprising 35 isolates with 6 subclusters with identical pairs, including similarity of clinical and environmental isolates (KpBHU50, EKpnBHU37 and EKpnBHU63; KpnBHU28, EKpnBHU21 and EKpBHU39; KpBHU34 and EKpnBHU3) and cluster III, IV, V, VI, VII and VIII consists of only 2, 4, 2, 2 and 1 isolates. When the two dendrograms of clinical and environmental origins were considered together for genotypically identical strains, it could be seen that identical strains KpBHU30 and EKpBHU14 did not have similar antibiograms. Similarly, in cluster-I, similar observations could be made in the case of identical strains KpBHU54, EKpBHU24 and EkpBHU56. In KpBHU31, KpBHU35 and KpBHU58; KpBHU50, EKpBHU37 and KpBHU63 had identical banding patterns. The Cluster-II consists of KpBHU50, EKpnBHU37 and EKpnBHU63; KpnBHU28, EKpnBHU21 and EKpBHU39; KpBHU34 and EKpnBHU3, had identical banding patterns but not similar antibiograms (Fig. 3,6,7).

S. No	Antibiotics	Resistance (environmental)	Resistance (clinical)	Amritsar (clinical)
1	Ampicillin	62.2	100	100
2	Amoxicillin + Clavulanic acid	48.7	100	95
3	Cefazolin/Cephalexin	98.3	100	NA
4	Cefuroxime	90.8	100	NA
5	Cefotaxime/Ceftriaxone	59.7	98.4	96
6	Gentamicin	24.4	96.9	89
7	Amikacin	47.1	91.5	85
8	Ciprofloxacin	36.9	99.2	96
9	Trimethoprim + Sulfamethoxazole	21.8	96.9	NA
10	Levofloxacin	38.6	99.2	NA
11	Piperacillin + Tazobactam	71.4	87.6	NA
12	Cefpirome/Cefepime	62.2	96.1	96
13	Imipenem	8.4	32.6	81
14	Ertapenem	52.9	86.8	89
15	Meropenem	6.7	72.1	NA
16	Colistin	0	15.5	16

Table 1: Resistance pattern of clinical and environmental isolates of *K. pneumoniae* using the disc diffusion method.

Antimicrobials	Overall resistance rate of *K. pneumoniae* to various antimicrobials in Asian countries (date 2005-2019)			Present study	
	Prevalence pattern	Heterogenicity test	p-value	Clinical	Environment
Amikacin	40.8 (31.9-50.4)	93.7	<0.001	91.5	47
Gentamicin	58 (49.2-66.3)	89.7	<0.001	96.9	24.4
Cefotaxime/Ceftriaxone	79.2 (68.0-87.2)	93.8	<0.001	98.4	59.7
Ciprofloxacin	59.8 (48.6-70.1)	96.4	<0.001	99.2	36.9
Levofloxacin	54.1 (36.0-71.2)	99.4	<0.001	99.2	38.6
Imipenem	65.6 (30.8-89.0)	99.6	<0.001	32.6	8.4
Meropenem	62.7 (31.1-86.2)	99.5	<0.001	72.1	6.7
Trimethoprim + Sulfamethoxazole	58.2 (35.5-77.9)	98.8	<0.001	96.9	21.8
Cefpirome/Cefepime	72.6 (57.7-83.8)	96.9	<0.001	96.1	62.2
Colistin	2.9 (1.8-4.4)	0	<0.001	15.5	NA

Table 2: Overall resistance rate of *K. pneumoniae* to various antimicrobials in Asian countries (date 2005-2019).

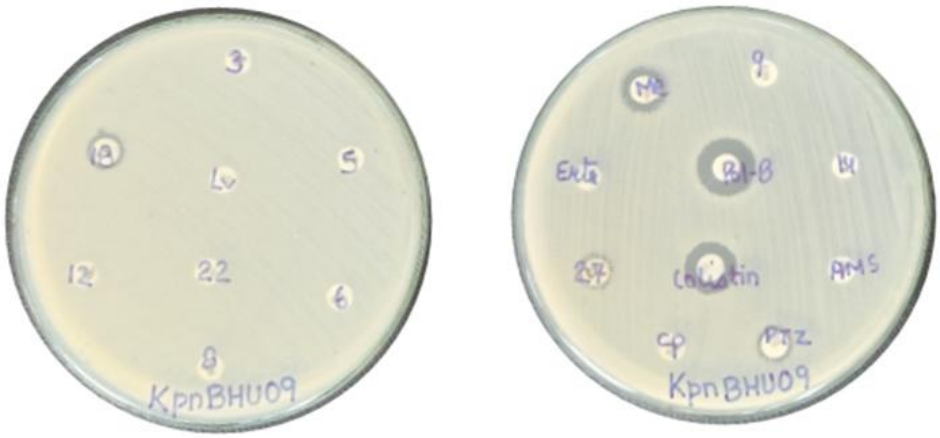

Figure 1: Antibiotics susceptibility testing (disk diffusion method) of clinical isolate of *K. pneumoniae*.

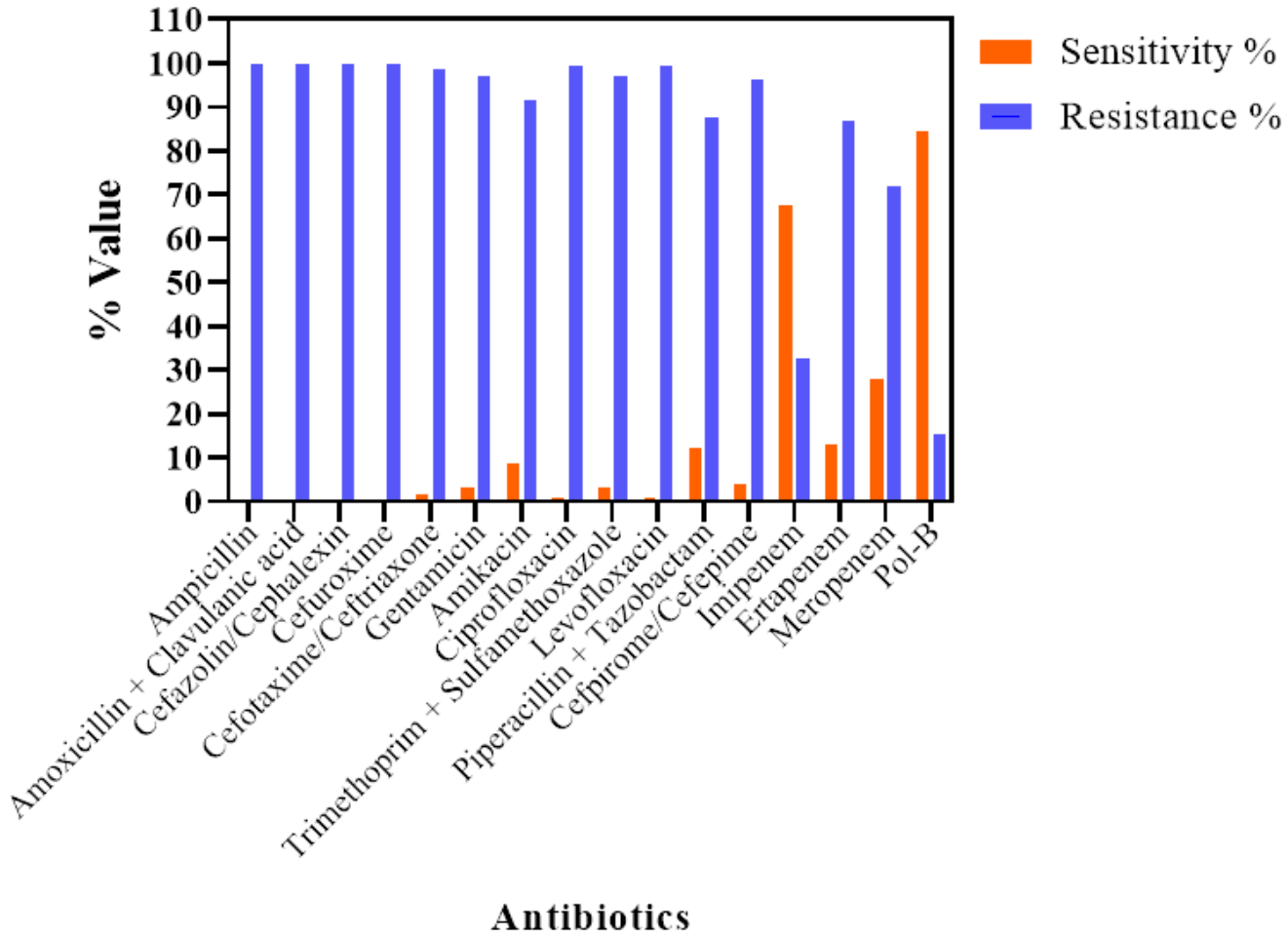

Figure 2: The bar diagram shows antibiotics drug resistance pattern of clinical isolates of *K. pneumoniae*.

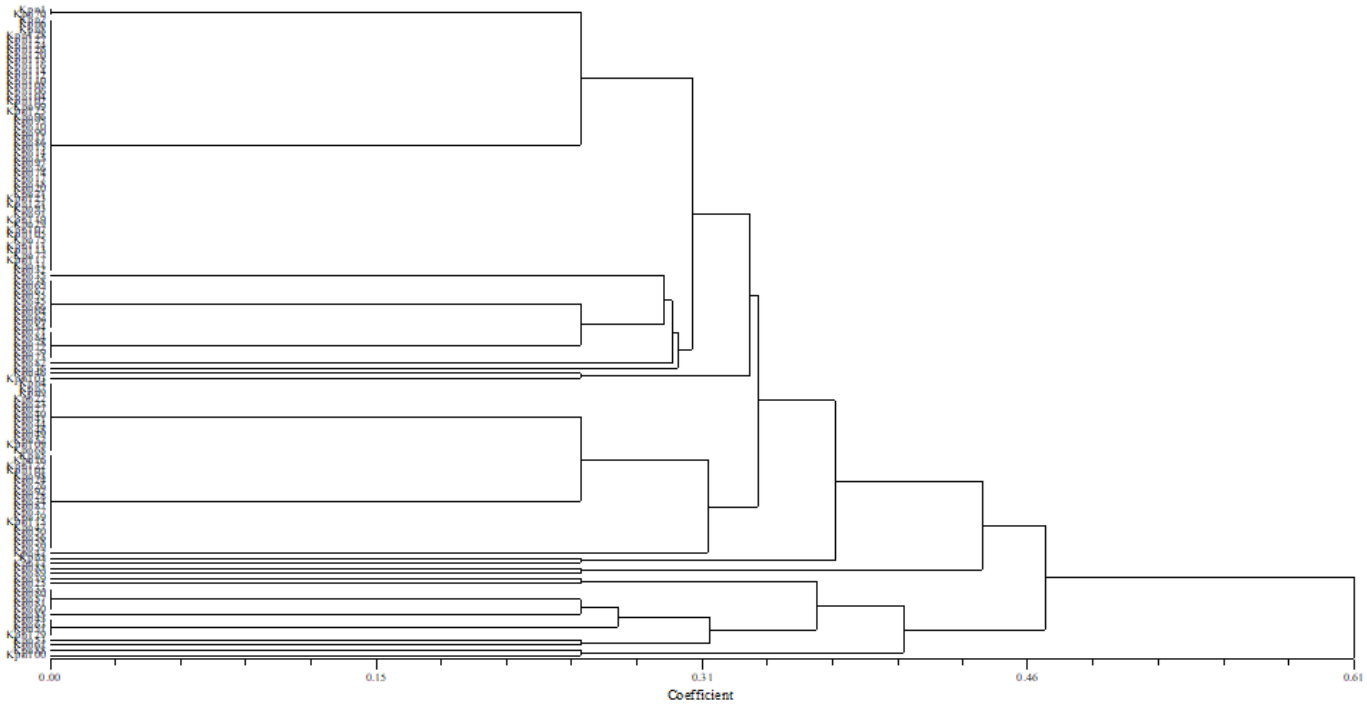

Figure 3: Dendrogram of clinical isolates of *K. pneumoniae* based on antibiotics susceptibility testing.

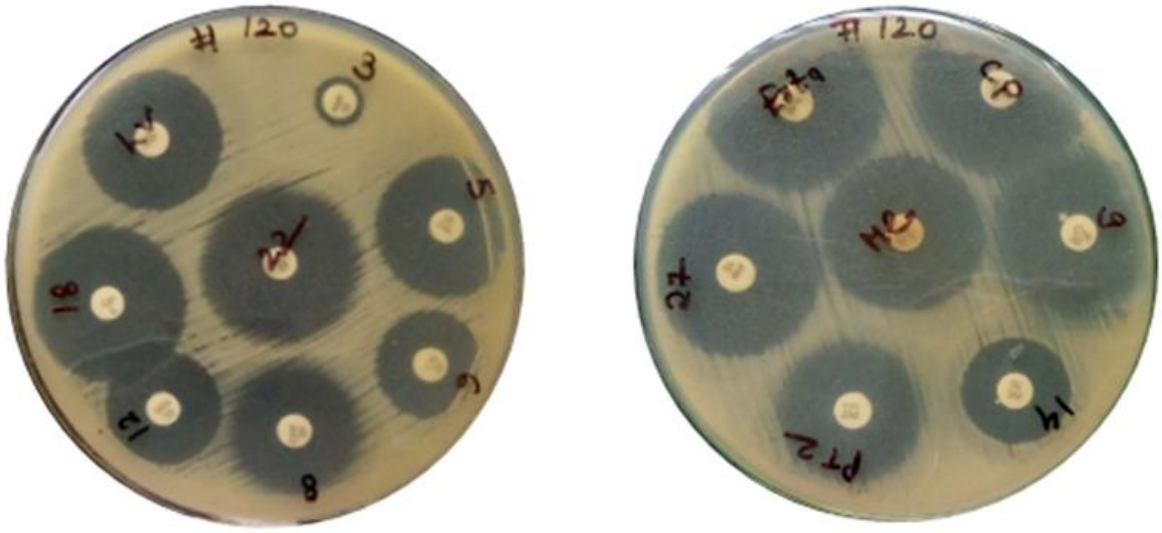

Figure 4: Antibiotics susceptibility test (disk diffusion method) of environmental isolate of *K. pneumoniae*.

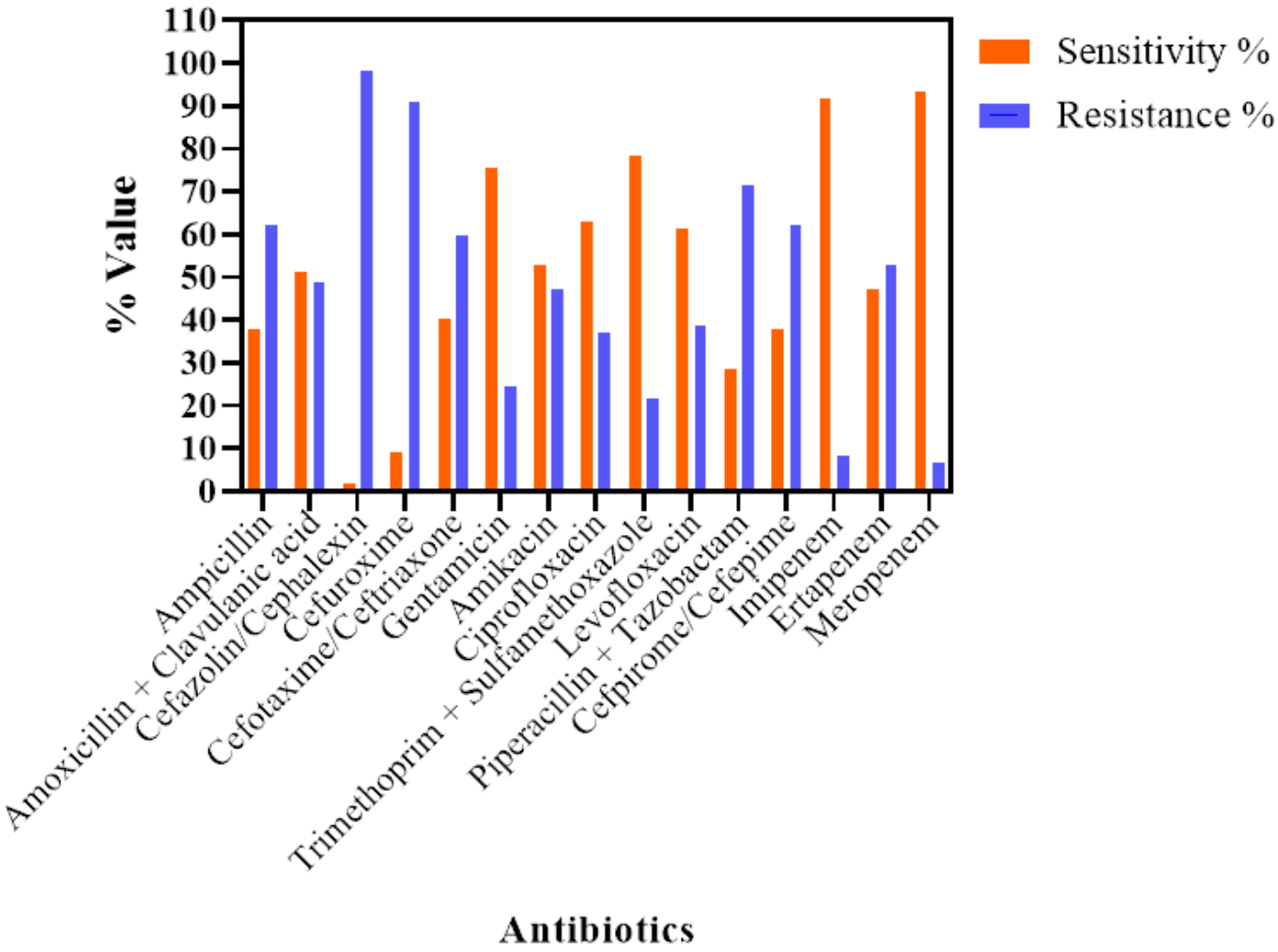

Figure 5: Bar diagram showing antibiotics drug resistance pattern of environmental isolates of *K. pneumoniae*.

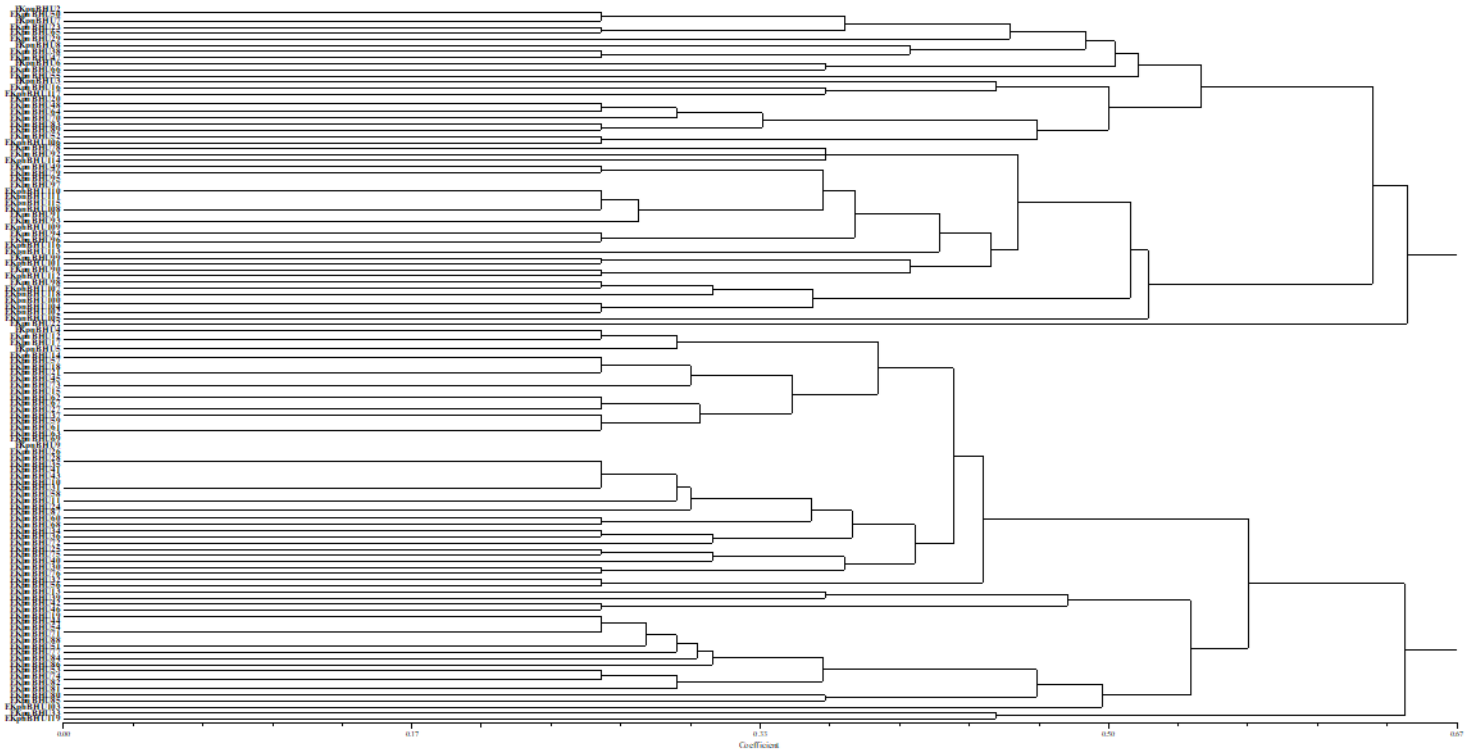

Figure 6: Dendrogram of environmental isolates of *K. pneumoniae* based on antibiotics susceptibility testing.

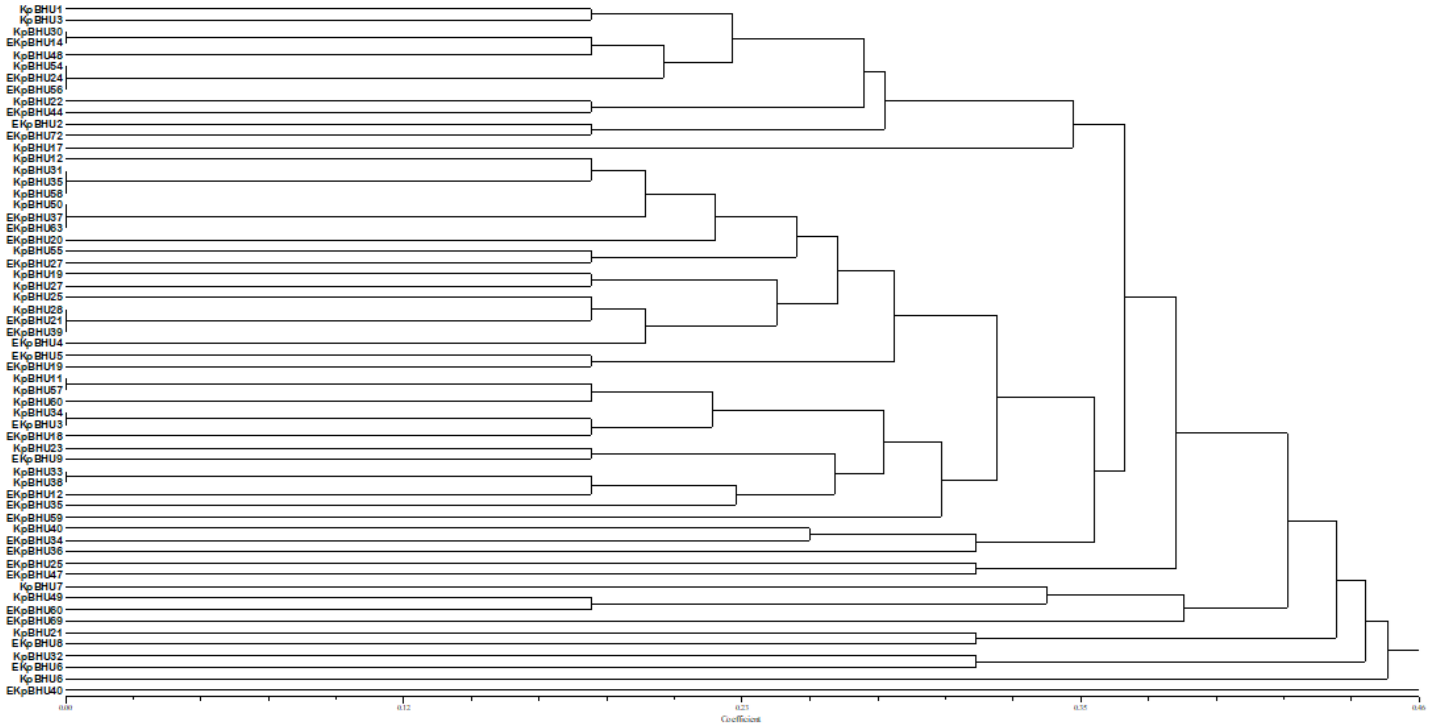

Figure 7: Dendrogram of *K. pneumoniae* based on ERIC-PCR of thirty randomly selected isolates of from clinical and environmental.

Discussion

The emergence and rapid spread of hypermucoviscosity carbapenem-colistin-resistant *K. pneumoniae* in healthcare settings is a serious concern worldwide. *K. pneumoniae* belongs to the ESKAPE group of microorganisms, especially known for the acquisition of antimicrobial resistance genes from environmental sources and also reflects the ability of ESKAPE microorganisms to escape

from killing by antimicrobial substances. Thus, it defies eradication by conventional antibiotic therapy, which accounts for extensive morbidity and mortality worldwide.

The clinical isolates were resistant at a very high level as compared to that of environmental, while 11 out of 16 antibiotics tested >90% of the clinical isolates expressed resistance while only two antibiotics, i.e., Cefuroxime and Cefazolin/Cephalexin were found to resist >90% of the environmental isolates. It is quite likely that the resistance mechanism in the environmental bacteria might be located on a chromosome, leading to intrinsic resistance [21].

On comparing the resistance pattern of clinical versus environmental isolates, it could be observed that all of the 129 (100%) clinical isolates were MDR, with 14.7% of them having PDR status. Contrary to this, only 10.8% of the environmental isolates could be designated MDR with nil PDR. Interestingly, 66.7% of the isolates were resistant to only the β-lactam group of antibiotics. This difference could be explained based on high selection pressure in the hospital environment leading to the accumulation of a variety of resistance genes in plasmids borne by the sturdy *K. pneumoniae* bacteria, which is incessantly between the natural environment and the hospital setup. Although not unexpected, the present observation made in this study is shown for the first time in the literature.

Interestingly, a study reported from North India during the same period, i.e., 2021-22, had a similar pattern and rate of prevalence against different antibiotics. Interestingly, there has been a rising pattern of drug resistance in *K. pneumoniae* from 2005 to 2019 (Table 2). It has almost reached a peak and Colistin remains the only ray of hope. The resistance against Colistin also has risen from 2.9% during 2005-2019 to 15.5% in the present study [22-31].

A review article entitled "*Klebsiella pneumoniae*: an increasing threat to Public Health" by Effah, et al., analyzed the 20 relevant articles published between 2005-2019 from different countries of Asia with a special focus on China [32]. Since there is a duration of 14 years, there are a lot of variations in drug resistance. When we compare the drug resistance of the present study, where the clinical samples were collected during 2021-2022, *K. pneumoniae* shows unusually high resistance rates against different antibiotics. These resistance rates indicate that before and during the year 2021-22, our hospital might have practiced indiscriminate use/misuse of antibiotics, except colistin, which was resisted by 15.5% of the isolates in the present study. However, previous studies from different countries also showed that the resistance rates vary from 1.8-4.4% (Table 2). The high heterogeneity index shows the huge variation in the different reports, which might have occurred due to different selection pressures over the 14 years and different antibiotics policies, if any, in different hospitals. The three Indian studies included in this review were also conducted in the years 2015 (33), 2012-14 (34) and 2014-2016 (35). Intriguingly, the study conducted recently had a similar resistance rate from North India (Amritsar) (21), whereas all the other antibiotics tested have a resistance rate ranging between 60-100% [21,33-35]. Interestingly, the resistance rate for colistin was 16%, while in the present study, it was 15.5%.

Based on the constructed antibiotic susceptibility pattern dendrogram, it was interesting to observe that 30 randomly selected clinical and environment isolates had different non-identical cluster patterns; however, 5 strains had similar sensitivity patterns. When the dendrogram was constructed based on the antibiotic susceptibility pattern and phage typing of the clinical isolates, the antibiotic susceptibility pattern had 8 small clusters comprising 2, 54, 10, 6,14, 19, 5 and 3 isolates having identical pairs. In contrast, the phage typing had only 3 identical pairs comprising 2 phages each. It means there is no correlation between the antibiotic and phage typing pattern. The lack of correlation between antibiotic typing and phage typing is due to the different mechanisms of inheritance of resistance genes, mainly plasmids, rather than the presence of various phage receptors on the bacterial surfaces.
High selection pressure may be appreciated by the dendrogram based on the resistance of clinical isolate, where 4 major clusters of identical strains could be seen. Interestingly, one cluster had 54 identical strains. On the contrary, the dendrogram prepared for environmental isolates on the same basis hardly shows the 3 or more identical strains in different branches.

The present study compares the resistance pattern in the isolates of both the origin, i.e., clinical and environmental. It shows that environment strains are only multi-drug resistant to a few antibiotics. However, high resistance rates in a few of the environmental isolates also depicted against β-lactam groups of antibiotics, especially Ampicillin, Cefotaxime, Cefpirome, Ciprofloxacin and Piperacillin, might be due to inherited chromosomal genes. However, the resistance rate of 71.4% in the

environmental strains needs explanation as, despite Tazobactam, the resistance is seen in these isolates. Do these isolates have mechanisms other than ESBL?

Based on ERIC PCR, the dendrogram prepared indicates that the resistance markers are primarily not located on chromosomes but on plasmids since whole genome analysis by ERIC PCR of both types of isolates shows rare identical strains, which is contrary to the dendrogram prepared based on drug resistance. Wyres and Kathryn, 2018 in a review article published in Current Opinion in Microbiology, emphasize that *K. pneumoniae* are intrinsically resistant to ampicillin due to penicillinase (SHV-1) in their chromosome [5,36-38]. They further suggested that additional drug resistance occasionally may arise through mutation in chromosomal genes and most AMR in *K. pneumoniae* results from the equisition of large and small consignations plasmids via horizontal gene transfer [7,39-41].

It is suggested that under the selection pressure of antibiotics in a clinical environment, the accumulation of AMR genes in a single strain results in pan-resistant strains [42]. Wyres and Holt have mentioned that clinical strains of *K. pneumoniae* can accumulate up to 400 AMR genes, followed by *A. baumannii* 278 and *E. cloacae* 277. They have depicted that *K. pneumoniae* can accumulate many plasmids ranging from 2-5 per strain. It has been shown that among the *Enterobacteriaceae* family, *K. pneumoniae* has the highest number (≈5500 genes) of genes per genome. Further, GC content per gene in *K. pneumoniae* is highly variable. All these inherent quality of *K. pneumoniae* enables it to accumulate the highest number of resistance genes in a single bacterial cell; added to this, unlike *E. coli*, *K. pneumoniae* can thrive well in environmental condition and also in the gut of human, pet animals, insects and aquatic animals. As the AMR genes primarily originate from the water and soil bacteria, which produce a spectrum of antibiotics for their protection, *K. pneumoniae* acquires these genes and brings them to the human gut as a key trafficker to disseminate in various clinically important bacteria. This is evident in the present study as many environmental strains were detected with resistance against many of the penicillin and cephalosporin groups of drugs, probably mediated intrinsically on chromosomes [37]. We could detect MDR strains of *K. pneumoniae* from the environment, while most of the clinical isolates were MDR and PDR [43].

Conclusion

It has been suggested that the gut microbiota of human beings only occasionally have *K. pneumoniae* as a commensal flora (6-10%). Therefore, *K. pneumoniae* may not be an essential component of the gut microbiota. When we combine all the above factors, we analyzed the *K. pneumoniae* position; it may be suggested that it is a key amplifier and spreader of AMR genes in the clinical environment. It may also be suggested that eradicating *K. pneumoniae* from the gut of human beings and from the clinical environment through the agency of bacteriophages can help us reduce the menace of antibiotic resistance.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This research received no specific grant from public, commercial or not-for-profit funding agencies.

Data Availability Statement

The original contributions in the present study are included in the article/supplementary material; further inquiries can be directed to the corresponding author.

Authors' Contribution

ANS: Conceptualization, Data curation, Formal analysis, Investigation, Validation, Visualization, Writing - original draft, Writing - review and editing. GN: Conceptualization, Data curation, Formal analysis, Investigation, Validation, Visualization, Writing - review and editing, Supervision, Project administration.

Acknowledgements

We are also grateful to Viral Research and Diagnostic Laboratory, DHR, Govt. of India, for providing the infrastructure for the current research work and the financial help as an incentive grant from IoE, Banaras Hindu University, given to GN.

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