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Frequency of Colistin Resistance Among the Common Gram- Negative Bacterial Pathogens; *Escherichia Coli, Klebsiella Pneumoniae, Acinetobacter Baumannii and Pseudomonas Aeruginosa*

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ABSTRACT

Objective: To determine the distribution and burden of Colistin resistant gram-negative bacterial isolates from clinical specimen.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from Jan 2019 to Jul 2020.

Methodology: All clinical samples (n=4199) received for bacterial culture during the study period were included in the study. Gram negative isolates including *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae* and *Escherichia coli* were tested for Colistin susceptibility using phenotypic methods, that included VITEK® 2 Systems Version: 08.01 (bioMerieux, France) and inhouse validated Colistin agar. The breakpoint of > 2 µg/ml was taken as resistant and ≤ 2 µg/ml as susceptible for all isolates.

Results: Overall, 309(7.4%), out of the total 4199 isolates were found to be resistant to Colistin. The resistance was highest in *Klebsiella pneumoniae* isolates, with 208(17.4 %) out of the total 1194 being found resistant in the study. The resistance among the isolates of *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Escherichia coli* was 47(5.5%), 33(2.8%) and 21(2.1%), respectively.

Conclusion: Resistance to Colistin is on the rise and emerging Colistin resistance is a challenge that needs urgent intervention in order to save this last line treatment option.

Keywords: Antimicrobial resistance, Colistin, Gram negative bacteria.

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INTRODUCTION

Polymyxins are currently considered the last line treatment options against extensively drug resistant gram-negative bacterial isolates.[1] Polymyxins are a cationic lipopeptide antibiotic that acts by disrupting the gram-negative outer membrane, of which only polymyxin B and polymyxin E (or Colistin) is in use for humans and due to the worldwide spread of extensively drug resistant (XDR) and pan drug resistant (PDR) bacteria, Colistin has become an important and at times the only available treatment option.[1,2] XDR isolates especially carbapenem resistant organisms (CROs) have become widespread in hospitals as the injudicious use of these antibiotics along with poor infection control practices has led to the selection and spread of the resistant bacterial population where polymyxins have now become our last line antibiotics against such CROs with chromosomal as well as plasmid-mediated resistance mechanisms being described for Colistin.[3] Reports of transferrable resistance to polymyxins in the last decade have raised serious concerns as plasmid-mediated transferrable resistance to Colistin is mediated through mobilized Colistin resistance-1 (mcr-1) gene which encodes an enzyme that catalyzes the addition of phosphorylethanolamine to phosphate groups in lipid A component of the bacterial outer membrane, first reported in 2015 in Escherichia coli isolates from food animals and Klebsiella pneumoniae from human isolates in China,[1,4] Later studies described various mcr-1 gene variants and seven more mcr gene families (mcr-2 to mcr-8),[1,5] also described an mcr-9 gene in Salmonella typhimurium in 2019.[6] There is a need to monitor emerging resistance to Colistin and employ necessary measures to limit the spread. The objective of this study was to determine the

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distribution and frequency of Colistin resistance among clinical isolates in our setup.

METHODOLOGY

The cross-sectional study was conducted at the Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from January 2019 to July 2020. Approval was obtained from the Institutional Review Board via letter FC-MIC 16-1/READ-IRB/22/1078.

Inclusion Criteria: Isolates of *E. coli, K. pneumoniae,* A. *baumannii* and *P aeruginosa* from clinical specimen including blood, cerebrospinal fluid, sputum, bronchoalveolar lavage, endobronchial washings, tracheal aspirates, urine, pus, tissue, and body fluids from patients of all ages and both genders, received for culture and sensitivity from the wards and intensive care units (ICU) of Combined Military Hospital (CMH) Rawalpindi, Pak-Emirates Military Hospital (PEMH) Rawalpindi, Armed Forces Liver Transplant Unit (ALTU) Rawalpindi, and Armed Forces Bone Marrow Transplant Center (AFBMTC), Rawalpindi, were included in the study.

Exclusion Criteria: Repeat specimens from the same patients were excluded.

This was a time-barred study and all clinical specimens received for culture and sensitivity during the study period were included using non-probability consecutive sampling technique. The samples were prepared in the microbiology laboratory using standard microbiological testing procedures.[7] Bacterial colonies were identified using gram staining, basic biochemical tests and respective API galleries (API E and API 20 NE) (bioMerieux, France). Colistin susceptibility testing was done using phenotypic methods that included VITEK® 2 Systems Version: 08.01 (bioMerieux, France) and inhouse validated Colistin agar using strengths of 2 µg/ml and 4g µ/ml using Muller-Hilton agar base (Oxoid, UK) and Colistin sulfate salt (Sigma-Aldrich, USA).[8,9] The breakpoint of > 2 µg/ml was taken as resistant and ≤ 2 µg/ml as susceptible for all isolates.[9] *Escherichia coli* NCTC 13846 and Escherichia coli ATCC 25922 were used as quality control strains. Compilation and statistical analysis of data was performed using Microsoft Excel and descriptive statistics were used to calculate frequencies and percentages.

RESULTS

Over eighteen months of the study period, a total of 4199 isolates from clinical specimen were tested for Colistin susceptibility. The distribution of clinical specimens included in the study is ilustrated in Figure-1.

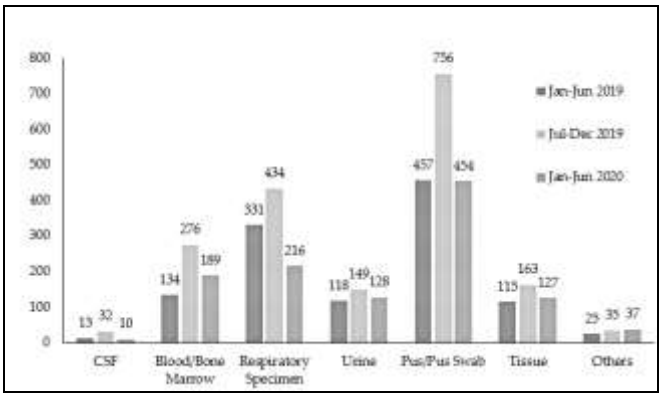

Figure-1: Year-wise Distribution of Clinical Specimen, (n=4199)

Out of the total isolates, 1194(28.4%) were *Klebsiella pneumoniae,* 1172(27.9%) were *Pseudomonas aeruginosa*, 983(23.4%) isolates were *Escherichia coli,* while 850(20.2%) were *Acinetobacter baumannii.* Our data shows that, overall, *K. pneumoniae* was the most frequent isolate followed by *P. aeruginosa.* The distribution of the isolates over the eighteen-month study period is given in Table.

Table: Year-wise Distribution of Number of Isolates (n=4199)

Duration	*Acinetobacter baumannii* (n=850, 20.2%)	*Pseudomonas aeruginosa* (n=1172, 27.9%)	*Klebsiella pneumoniae* (n=1194, 28.4%)	*Escherichia coli* (n=983, 23.4%)	Total (n=4199)
Jan - Jun 2019	243(20.4)	351(29.4)	304(25.5)	295(24.7)	1193
Jul - Dec 2019	380(20.6)	514(27.9)	556(30.1)	395(21.4)	1845
Jan - Jun 2020	227(19.6)	307(26.4)	334(28.8)	293(25.2)	1161

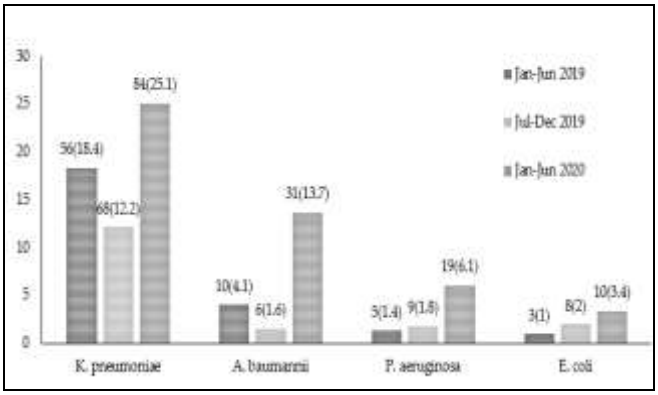

Figure-2: Year-wise Percentage of Colistin Resistance (n=4199)

The overall Colistin resistance among the four commonly isolated gram-negative bacteria was 309(7.4%). Resistance to Colistin was highest in *K. pneumoniae* i.e., 208(17.4%), followed by *A. baumannii* 47(5.5%), *P. aeruginosa* 33(2.8%) and E. coli 21 (2.1%).

The year-wise distribution of Colistin resistant isolates is shown in Figure-2, where an increasing trend can be noted in 2020.

DISCUSSION

The spread of Colistin resistant isolates is an emerging threat to public health as these infections not only contribute to higher mortality, but also higher treatment costs and prolonged hospital stay with the emergence of transferrable mechanisms of resistance leading to worldwide dissemination of resistant isolates as these resistance genes have also been reported to coexist with other resistance genes like carbapenemases, further limiting treatment options.[10] In our study, the Colistin resistant isolates were also resistant to multiple antibiotic classes. One study estimated the global burden of Colistin resistance from the mcr genes detected from human, plant and animal sources, to be from 45 countries with a combined prevalence of 4.7%.[1] In our study, the overall resistance was 7.4% among the four most commonly isolated gram-negative rods. Another study found 6.4% Colistin resistant gram-negative bacteria among isolates from blood cultures which is comparable to our results.[11] A local study reported similar findings with a frequency of 15.9% among Carbapenem-resistant Enterobacteriaceae (CRE) with the highest frequency in K. pneumoniae isolates.[12] K. pneumoniae had the highest frequency of Colistin resistance in our study at 17.4%, a finding consistent with another similar study which reported 17.3% resistance rates.[11] Contrarily, some studies have reported lower resistance rates as compared to the present study (7.7% and 4.6%),[13,14] with systematic reviews estimating the global trends of Colistin resistance being reported from Asian countries,[1,14] as Colistin resistance in A. baumannii in our study was 5.5% but higher resistance has been reported in another regional study with 9.6%,[15] with lower resistance reported from European countries.[16] Emerging Colistin resistance will make treatment even more challenging for physicians.[17] While there is a lack of reliable surveillance data from our region, another possible reason for the paucity of reliable literature, especially from resource poor countries, may be due to lack of simple phenotypic susceptibility testing methods for Colistin. Guidelines for testing and reporting Colistin have changed in recent years as prior to 2020, only broth microdilution was recommended as the standard testing method both by EUCAST and CLSI 7, 8 but in 2020, CLSI also included the Colistin agar test (agar dilution) and Colistin broth disk elution test and also revised the breakpoints for reporting, eliminating the susceptible category, as MIC of ≤ 2 µg/ml is now reported as intermediate.[18] For this study, the authors used a > 2 µg/ml was taken as resistant and ≤ 2 µg/ml as susceptible for all isolates in line with the EUCAST recommendations.[8] E. coli, K. pneumoniae, A. baumannii, and P. aeruginosa are all frequently isolated pathogens from hospitalized patients as these isolates are notorious for causing outbreaks in hospitals owing to their ability to colonize surfaces and medical devices.[19-22] An increasing Colistin resistance among these isolates is a cause of concern, as we are already over-reliant on it. Till the time newer and effective treatment options become available, Colistin is our last line treatment.

LIMITATIONS OF STUDY

The study's limitations include its single-center, descriptive design, which, while useful for reporting local resistance patterns, limits the generalizability of the findings to other populations or healthcare settings. Furthermore, the reliance on phenotypic methods alone for detecting Colistin resistance means that underlying resistance mechanisms were not characterized, leaving a gap in the understanding of the molecular epidemiology of the resistance observed.

CONCLUSION

The emerging Colistin resistance among these isolates is a challenge that needs urgent intervention at all levels from limiting the use in the animal farming industry, active nationwide surveillance, strict adherence to infection control guidelines, and an effective antimicrobial stewardship program in hospitals.

Conflict of Interest: None.

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Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

AI & IAM: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

MS & WH: Conception, data analysis, drafting the manuscript, approval of the final version to be published.

UK & UK: Data acquisition, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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