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The Prevalence of Carbapenem and Colistin-Resistant Gram-Negative Bacteria Isolated from Hospital-Admitted Patients with Bacteremia

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ABSTRACT

Carbapenem-resistance, a global health concern augmented with resistance against last-resort antibiotic colistin has become a great challenge. Bacteremia caused by Carbapenem-resistant (CR) Gram-negative bacteria (GNB) has been linked to prolonged hospitalization and high mortality. Colistin-resistant *Acinetobacter baumannii* (CoRAB) represent a serious threat to patients admitted to Intensive Care Unit (ICU) with mortality estimates up to 100%. This study aimed to estimate the current burden of CR and CoR GNB in admitted patients. In this prospective study, a total of 21,600 blood cultures were processed in automated BACTEC system. Conventional methods and Automated Profile Index (API) 20E and 20NE were used for GNB identification. Carbapenem and colistin Antimicrobial Susceptibility Testing (AST) was determined using disc-diffusion and broth microdilution (BMD) methods respectively. The pooled CR was 25.59%. CR was highest in *Klebsiella spp.* (44.2%) and *Acinetobacter baumannii* (34.66%). The overall CoR among 2903 GNB was 0.96% while among 743 CR-GNB was 3.76%. CoR rates are lower in Sindh compared to Punjab. CoR has reached up to 5.20% in *Klebsiella spp.* and up to 3.8% in *A. baumannii.* All *Pseudomonas aeruginosa* isolates were sensitive to colistin. Significant proportion of CoRKP and CoRAB in ICU alarms the situation and calls for to seek ways to minimize the emergence of CoR. *Klebsiella spp.* and *A. baumannii* remain the predominant CR and CoR GNB in Bloodstream infections (BSI). Presence of CoR-*E. coli* in pediatric wards highlight the poor hygienic practices and fecal transmission.

Keywords: Blood cultures, Broth microdilution method, Carbapenem resistance, Colistin resistance, Gram-negative bacteria

INTRODUCTION

Carbapenems (β-lactams) are the choice of treatment for multi-drug resistant (MDR) Gram-negative bacteria (GNB). Carbapenem widespread use resulted in Carbapenem-resistance (CR) which is a major global health concern. There has been a growing incidence of bloodstream infection (BSI) caused by CR-GNB among hospitalized patients (Gondal et al., 2023). Intrinsic CR is conferred by structural differences in the cytoplasmic membrane or lack of a particular target. For acquired resistance, the major mechanism is the carbapenemases production encoded either in the chromosome or in the plasmid. A carbapenemase enzymatically hydrolyze the carbapenem antibiotic and type of carbapenemase varies with the geographical location and gene expression in the organism. The second mechanism includes antibiotic efflux or poor penetration of antibiotic into the bacterial outer membrane. The third includes target-site modification through genetic mutation or post-translational modification (Aurilio et al., 2022). World Health Organization (WHO) in 2017 issued a priority list of most-resistant bacteria. Some of these bacteria are intrinsically resistant to carbapenems others have acquired resistant mechanisms(Aurilio et al., 2022). Of particular concern are Carbapenem-resistant Acinetobacter baumannii (CRAB) (Jiang et al., 2022) and Carbapenem-resistant Klebsiella pneumoniae (CRKP) (Wu, Zheng, & Yao, 2022). Bacteremia caused by CR-GNB has been linked to prolonged hospitalization and an elevated risk of mortality (Jean, Harnod, & Hsueh, 2022). Despite the early reports concerning the adverse neurotoxic, nephrotoxic effects noted polymyxins efficacious in treating bacteremia among

neonates (Ambreen et al., 2020). Furthermore, the adverse effects were found to be moderate and reversible among patients with impaired renal functions (Babar, Dodani, & Nasim, 2021). This have made a comeback of narrow spectrum polymyxin B and polymyxin E (colistin) as a last-resort treatment option for CR-GNB. The increased and injudicious use soon resulted in Colistin-resistance (CoR). Modification or complete loss of lipopolysaccharide (LPS) (Novović & Jovčić, 2023) and mutations in the plasmid-mediated *mcr* gene are the common mechanisms for CoR (Hameed et al., 2019). As treatment with polymyxin itself can contribute to CoR, one proposed approach is the combination therapy carbapenem with polymyxin (Dickstein et al., 2020). The reference standard for polymyxin AST according to Clinical Laboratory Standard Institute (CLSI) is broth microdilution (BMD) method. BMD is a labor-intensive test and costly (Yusuf, van Westreenen, Goessens, & Croughs, 2020). The actual burden-estimates of CoR through BMD is limited in Pakistan. The minimum duration for positive blood culture is 2-3 days, for this reason empirical treatment mostly relies on knowledge of likely pathogen and their local susceptibility pattern. Inappropriate empirical treatment is associated with significant mortality among patients with CR-GNB (Babar et al., 2021). In this study, we report the current burden of CR and CoR GNB isolated from admitted patients in a tertiary care hospital of Karachi, Pakistan.

MATERIALS AND METHODS

Study duration and area: This prospective, cross-sectional study from January 2022 to June 2023 was conducted at the Microbiology Laboratory of Sindh Institute of Urology and Transplantation (SIUT) in Karachi, Pakistan. SIUT is a 700-bed urban tertiary care hospital and caters mostly to patients for renal diseases, dialysis and transplantation. It also caters for general surgery, oncology, and gastroenterology patients.

Specimen collection and evaluation: Venous blood samples from admitted patients were aseptically obtained in BACTEC™ bottles and processed in an automated BACTEC system. No discrimination was made on gender, age, immunocompetent or immunocompromised basis. Contaminated samples, outpatients and organisms intrinsically resistant to polymyxins were excluded.

Identification of Gram-negative bacteria: Each BACTEC-positive blood culture bottle was initially examined by Gram staining of the broth and subsequently sub-cultured on Chocolate, Blood and MacConkey's agar plates. Plates were incubated for 18-48 hours aerobically at 37°C. Bacteria were preliminary identified using conventional methods: microscopy, colony morphology,Gram's staining, pigmentation, motility, and swarming. *Enterobacterales* and other GNB were traditionally identified using biochemical tests: triple sugar iron, citrate utilization, urease, indole, DNase and H_2S production and rapid tests: catalase, coagulase and oxidase (Munir et al., 2021).

Automated profile index (API) (bioMerieux): The representative MDR GNB were further confirmed using API 20E and 20NE tests strips. API test strips were processed according to manufacturer's instructions. API system utilizes 21 miniaturized biochemical tests and a computer database which efficiently differentiate members of *Enterobacteriaceae* and other GNB.

Antimicrobial sensitivity testing (AST): AST was performed on Mueller Hinton agar using Kirby-Bauer method. Common antibiotics for the treatment of GNB were used. Zone of inhibition around the discs were interpreted as per CLSI guidelines. MDR-GNB were further assessed for CR using discs of Imipenem (IPM) or Meropenem (MEM)(Munir et al., 2021).

Minimum Inhibitory Concentration (MIC): All CR-GNB were checked for colistin MIC using broth microdilution (BMD) method. Dilution ranges (0.5-16 µg/ml) were prepared using Cation-adjusted Muller-Hinton broth (Oxoid, UK) and Colistin sulfate (Sigma-Aldrich, USA). Bacterial suspensions (0.5 MacFarland) from freshly grown bacteria were added into microtube plates.The microtube plates were incubated for 24 h at 37°C in ambient air. The breakpoints of the CLSI (M100-Ed-33) guidelines were used for the results interpretation. (MIC ≤2 µg/ml were considered Intermediate and MIC ≥4 µg/ml as Resistant). *Escherichia coli* ATCC 25922 strain and *Morganella morganii* strain (intrinsically resistant to colistin) were used as controls**.**

Statistical analysis: Data was stored in Microsoft Excel and analysis was performed using Statistical Package for the Social Sciences (SPSS) version 20.

RESULTS

A total of 21,600 blood culture specimens were collected from admitted patients during January-2022 to June-2023. Of total specimens, 32% were positive for bacterial pure culture and the prevalence of GNB was 14% (n=2903), mostly from pediatric 43% (n=1248) and surgical wards 34% (n=987) followed by ICU patients 15% (n=435) and other wards 8% (n=233)**.** The common GNB isolated were *Escherichia coli* 31% (n=909), *Klebsiella pneumoniae* 27% (n=782), *Acinetobacter baumannii* 21% (n=600) and *Pseudomonas aeruginosa* 16% (n=464) followed by other GNB including *Morganella morganii* 1.5% (n=44), Typhoid Salmonella 1.24% (n=36), *Proteus spp.*1.10% (n=32), *Aeromonas hydrophila* 0.75% (n=22), *Enterobacter aerogenes* 0.27% (n=08) and *Citrobacter freundii* 0.20% (n=06).

AST of 2903 GNB showed 25.59% (n=743) isolates were resistant to carbapenem (meropenem or imipenem), consisting of *Klebsiella pneumoniae* (n=346), *Acinetobacter baumannii* (n=208), *Escherichia coli* (n=151) and *Pseudomonas*

aeruginosa (n=38). *Klebsiella spp.* showed the highest CR (44.2%) followed by *A. baumannii* (34.66%), *E. coli* (16.61%) and *P. aeruginosa* (8.18%). Predominant number of CR-GNB were isolated from ICU patients 53.02% (n=394) followed by surgical wards 18.84% (n=140), pediatric wards 15.61% (n=116) and other wards 12.51% (n=93). Majority of the CR-*E. coli* were isolated from ICU and pediatric wards while CRKP and CRAB from ICU patients. **Figure 1**

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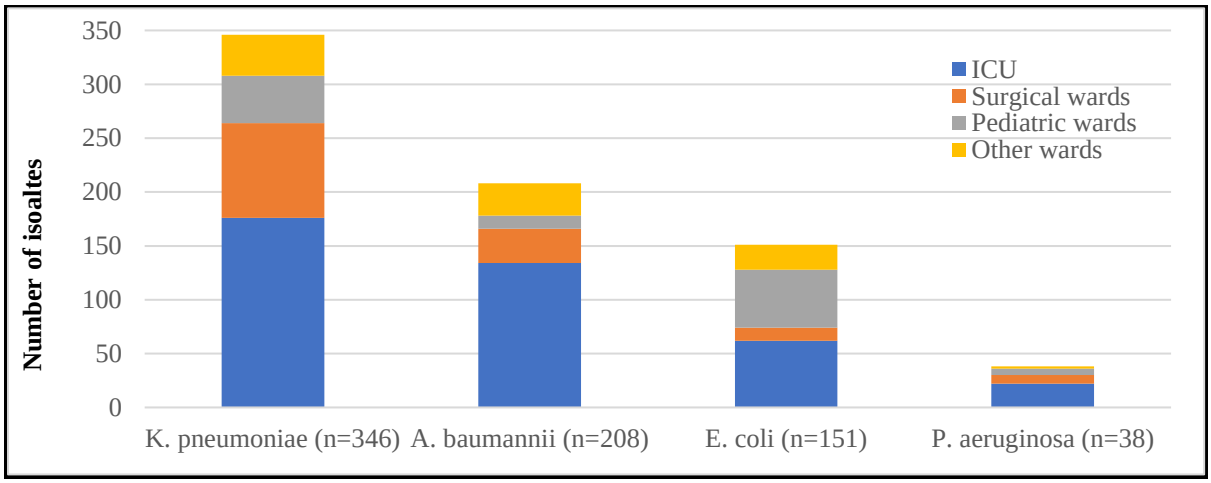

Figure 1. Prevalence of blood-isolated Carbapenem-resistant GNB in different hospital wards

Among the 743 CR-GNB, 28 (3.76%) isolates were colistin-resistant using MIC through broth microdilution method. Majority of *A. baumannii* (48.55%) exhibited colistin MIC 1µg/ml while more than half of CoRKP (55.5%) and CoRAB (62.5%) revealed MIC ≥16 µg/ml. Highest number of *K. pneumonia* and *A. baumannii* showed MIC in the range of 1 µg/ml, *E. coli* in the range of 0.5 µg/ml and *P. aeruginosa* in the range of 1-2 µg/ml **Table 1.**

Table 1. Colistin MIC of 743CR-GNB isolates

MIC range	0.5 µg/ml	1 µg/ml	2 µg/ml	4 µg/ml	8 µg/ml	16 µg/ml	Colistin resistance
***K. pneumoniae* (n=346)**	108 (31.21%)	118 (34.10%)	102 (29.47%)	2 (0.57%)	6 (1.73%)	10 (2.89%)	18 (5.20%)
***A. baumannii* (n=208)**	38 (18.2%)	101 (48.55%)	61 (29.32%)	1 (0.48%)	2 (0.96%)	5 (2.40%)	08 (3.8%)
***E. Coli* (n=151)**	77 (50.99%)	38 (25.16%)	34 (22.51%)	1 (0.66%)	0 (0%)	1 (0.66%)	02 (1.32%)
***P. aeruginosa* (n=38)**	5 (13.15%)	16 (42.10%)	17 (44.73%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Highest rate of CoR was exhibited by *K. pneumoniae* 5.20% followed by *A. baumannii*3.8% and *E. coli* 1.32%. All isolates of *P. aeroginosa* were sensitive to colistin. Most of the CoRKP were isolated from ICU and surgical wards while majority the CoRAB were recovered from ICU. Presence of the significant proportion of CoRKP and CoRAB in ICU wards alarms the injudicious and frequent use of colistin for critically ill patients. All CoR-*E. coli* were isolated from pediatric wards indicating the unhygienic hand practices and fecal contamination. **Figure 2.**

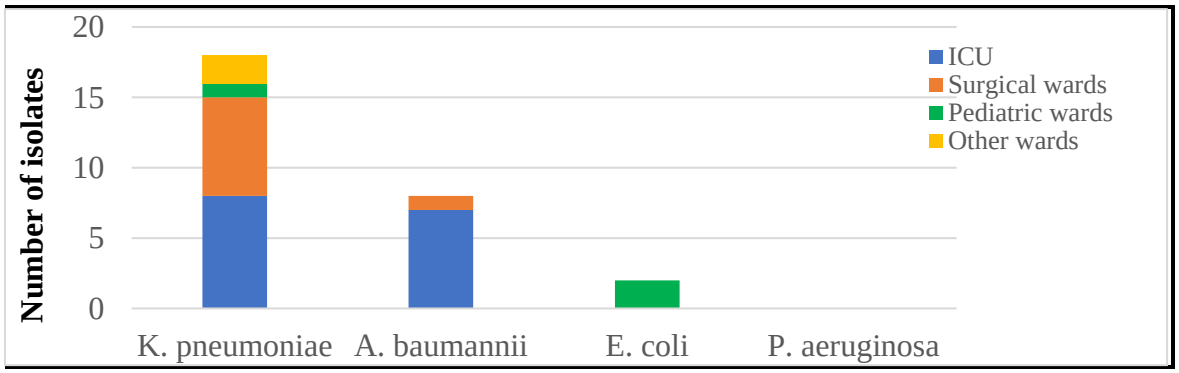

Figure 2. Prevalence of blood-isolated Colistin-resistant GNB in different hospital wards

DISCUSSION

A total of 21,600 blood culture specimens were collected from admitted patients during January-2022 to June-2023. Overall positiveness for bacterial pure-culture was 32% and for GNB was 14%. The positive incidence rate (32%) in our study population is significantly higher compared to previous studies 11.9% and 19.6% (Ambreen et al., 2020; Munir et al., 2021). The reason might be we included only admitted patients which frequently undergo invasive-surgical procedures and the ratio of bacteremia has been suggested higher among admitted patients in several studies (Fatima et al., 2023). We found the isolation rate of GPC was higher (18%) compared to GNB (14%) which is in contrast to previous studies where they reported higher frequency of GNB compared to GPC (Ambreen et al., 2020; Inam, Ikram, Malik, Jabeen, & Saeed, 2023). Likely explanation is, we found many of the GPC were Coagulase-negative Staphylococcus (CoNS) which are considered most common contaminants of blood cultures (Bhosle, Thakar, & Modak, 2022).

In this study, the four predominant GNB causing bacteremia were *E. coli*, *K. pneumoniae*, *A. baumannii* and *P. aeruginosa*. Our results are similar to a previous study from our center where they reported *Klebsiella spp*. bacteremia (27%) (Badlani, Dodani, Nasim, Babar, & Azmi, 2020). Comparable bacterial prevalence has been reported in another study (Santimaleeworagun et al., 2020). One study reported *P. aeruginosa* and *S. typhi* the predominant cause of BSIs in pediatric ICU followed by *E. coli* and *Klebsiella* (Ayaz, Hameed, Amber, & Zafar, 2020). Another study from Pakistan reported the predominant cause of bacteremia *S. typhi*, *E. coli*, *S. aureus*, *K. pneumonia*, and *Enterococcus* species. The variation in dominant organisms can be explained, we mostly cater patients for renal diseases in our hospital and these patients frequently acquire urinary tract infections (UTIs) which have been suggested the significant source (77%) of BSIs (Dodani, Nasim, Aziz, & Naqvi, 2020).

In this study, we report overall carbapenem resistance 25.59%. The CR burden is comparatively low in our province compared to 42.1% in Punjab (Gondal et al., 2023). We found CR only among four GNB groups which include *K. pneumoniae*, *A. baumannii*, *E. coli* and *P. aeruginosa*. CR was highest among *Klebsiella spp* (44.24%) and *A. baumannii* (34.66%). *Klebsiella spp*. remain the most dominant CR enterobacterale in BSIs (Fatima et al., 2023). Recently, the prevalence of CRKP has been reported 38% (Imtiaz, Syed, Rafaque, Andrews, & Dasti, 2021). *A. baumannii* is an uncommon BSIs pathogen, very often MDR (Kern & Rieg, 2020) and is a major concern of mortality primarily with respiratory tract infections in ICU (Jiang et al., 2022). Limited data regarding CRAB is available from Sindh, few studies from Punjab report very high prevalence of CRAB 84% (Ahsan et al., 2022) and >80% (Jabeen et al., 2022). The Global Antimicrobial Surveillance System (GLASS) has estimated >50% of CRAB among hospitalized patients. We estimated 16.61% *E. coli* and 8.18% *P. aeruginosa* resistant to carbapenems. The overall rates of CR *Pseudomonas aeruginosa* among elderly inpatients has been estimated 25.8% in China (Qin et al., 2022). The pooled rates of resistance to carbapenem in *E. coli* were estimated 5.0% in Iran (Nasiri et al., 2020). In many studies, *E. coli* is the first pathogen in number causing BSIs while *P. aeruginosa* is among the top three causing mortality (Kern & Rieg, 2020).

In our study, majority of the CRKP and CRAB were isolated from ICU patients while CR-*E. coli* from ICU and pediatric wards. This is in comparison with several studies where they report *Klebsiella* spp. and *A. baumannii* are the most notorious nosocomial pathogens harbouring a number of infectious plasmid-mediated resistance genes and are frequently associated with ICU and immunocompromised patients (Moubareck & Halat, 2020)

In this study, the pooled colistin-resistance (CoR) rate among the 2903 GNB was 0.96% while among 743 CR-GNB was 3.76%. CoR rates are lower in Sindh compared to Punjab where they reported 10.46% enterobacterales were resistant to polymyxins (Furqan et al., 2022). The global pooled prevalence of CoR was recorded 3.1% in 2022 (Uzairue et al., 2022). In our study, the highest rate of CoR was exhibited by *K. pneumoniae* 5.20%. Again this is in contrast with Punjab where they reported CoR three-fold (15%) among *Klebsiella* spp. (Imtiaz et al., 2021). With these findings, we hypothesize that the Punjab province is the source-emergence of CoR strains and frequent travelling from Punjab to other provinces has resulted in the dissemination of CoR over the country. Globally, the highest rate of CoR in *K. pneumoniae* have been recorded in Thailand (19.2%) and lowest (0.8%) in South Korea (Uzairue et al., 2022). The second dominant organism resistant to colistin was *A. baumannii* (3.8%) in our study. CoRAB represent a serious threat to critically ill patients as mortality rate has been estimated (100%) among 13 patients who had CoRAB BSI (Papathanakos et al., 2020). The highest rate of colistin resistance in *A. baumannii* have been recorded from Lebanon (17.5%) and China (12%) (Pormohammad et al., 2020). The pooled prevalence of CoRAB in five hospitals of Lahore have been estimated 7.3% (Ahsan et al., 2022). Compared to Sindh, the pooled CoRAB burden is two-fold in Punjab. In our study, 1.32% of *E. coli* showed CoR. A slightly higher CoR rate for *E. coli* (2.9%) has been estimated in Thailand

(Santimaleeworagun et al., 2020). In our study, all *P. aeroginosa* isolates were sensitive to colistin which is in contrast to a study where they reported CoR among *P. aeruginosa* 1.6% (Santimaleeworagun et al., 2020). Most of the CoRKP were isolated from ICU and surgical wards while majority the CoRAB were recovered from ICU. Presence of the significant proportion of CoRKP and CoRAB in ICU wards alarms the injudicious and frequent use of colistin for critically ill patients. All CoR *E. coli* were isolated from pediatric wards indicating the unhygienic hand practices and fecal contamination.

CONCLUSION

The overall carbapenem resistance rate 25.59% indicate that carbapenems can be used as empirical treatment for MDR-GNB infections. The CR and CoR burden is low in Sindh compared to Punjab. *Klebsiella spp.* and *A. baumannii* are the leading carbapenem and colistin resistant pathogens causing infections in admitted patients with bacteremia.

CONFLICT OF INTEREST

The authors declared no conflict of interest

AUTHOR'S CONTRIBUTION

RMAK: Study conception and design, data acquisition and analysis, interpretation, drafting, critical revision, final approval. AN: Data analysis, data acquisition, drafting, critical revision, interpretation, and final approval. MSI: Data analysis, data acquisition, critical revision, final approval. FM: Data analysis, interpretation, drafting, critical revision, final approval. TK: Data acquisition, Data analysis, interpretation, final approval. AA: Data analysis, interpretation, critical revision, final approval. MM: Data analysis, drafting, interpretation, critical revision, final approval. SKD: Data analysis, critical revision, drafting, interpretation, final approval

REFERENCES

Ahsan, U., Mushtaq, F., Saleem, S., Malik, A., Sarfaraz, H., Shahzad, M., Ahmad, I. (2022). Emergence of high colistin resistance in carbapenem resistant Acinetobacter baumannii in Pakistan and its potential management through immunomodulatory effect of an extract from Saussurea lappa. *Frontiers in pharmacology, 13*, 986802.

Ambreen, G., Salat, M. S., Hussain, K., Raza, S. S., Ali, U., Azam, I., . . . Fatmi, Z. (2020). Efficacy of colistin in multidrug-resistant neonatal sepsis: experience from a tertiary care center in Karachi, Pakistan. *Archives of disease in childhood*.

Aurilio, C., Sansone, P., Barbarisi, M., Pota, V., Giaccari, L. G., Coppolino, F., . . . Pace, M. C. (2022). Mechanisms of action of carbapenem resistance. *Antibiotics, 11*(3), 421.

Ayaz, I., Hameed, H., Amber, W., & Zafar, T. (2020). Nosocomial Blood Stream Infections in Pediatric Intensive Care Unit of Fauji Foundation Hospital, Rawalpindi Pakistan. *Journal of Islamabad Medical & Dental College, 9*(4), 269-274.

Babar, Z. U., Dodani, S. K., & Nasim, A. (2021). Treatment outcome and adverse effects of colistin in adult patients with carbapenem-resistant gram-negative bacteremia from Pakistan. *International Journal of Infectious Diseases, 106*, 171-175.

Badlani, S., Dodani, S. K., Nasim, A., Babar, Z., & Azmi, M. (2020). Trends in Antimicrobial Resistance Pattern of Klebsiella Bacteremia over a 6 Years Period (2010-2015) in a Hemodialysis Centre in Pakistan. *Infectious Diseases Journal of Pakistan, 29*(2), 27-30.

Bhosle, P. S., Thakar, V. H., & Modak, M. (2022). Coagulase Negative Staphylococcus Species Isolated from Blood Culture: A Pathogen or a Contaminant? *Natl J Lab Med., 11*(4), 9-MO05.

Dickstein, Y., Lellouche, J., Schwartz, D., Nutman, A., Rakovitsky, N., Dishon Benattar, Y., . . . Durante-Mangoni, E. (2020). Colistin resistance development following colistin-meropenem combination therapy versus colistin monotherapy in patients with infections caused by carbapenem-resistant organisms. *Clinical Infectious Diseases, 71*(10), 2599-2607.

Dodani, S., Nasim, A., Aziz, T., & Naqvi, S. A. (2020). Gram Negative Blood Stream Infection In Renal Transplant Recipients: Risk Factors and Outcome from A Transplant Center In Pakistan. *Transplantation, 104*(S3), S66.

Fatima, M., Dodani, S. K., Babar, Z. U., Badlani, S., Rani, B., Mushtaq, M., Nasim, A. (2023). Outcome, risk factors and therapeutic strategies in carbapenem-resistant Gram-negative bacteraemia from Pakistan. *JAC-Antimicrobial Resistance, 5*(3), dlad076.

Furqan, W., Ali, S., Usman, J., Hanif, F., Naeem, A., Nasrullah, A., & Tayyab, N. (2022). Assessing Colistin Resistance by Phenotypic and Molecular Methods in Carbapenem-resistant Enterobacterales in a Tertiary Care Hospital in Pakistan. *Infection and drug resistance*, 5899-5904.

Gondal, A. J., Choudhry, N., Bukhari, H., Rizvi, Z., Jahan, S., & Yasmin, N. (2023). Estimation, Evaluation and Characterization of Carbapenem Resistance Burden from a Tertiary Care Hospital, Pakistan. *Antibiotics, 12*(3), 525.

Hameed, F., Khan, M. A., Muhammad, H., Sarwar, T., Bilal, H., & Rehman, T. U. (2019). Plasmid-mediated mcr-1 gene in Acinetobacter baumannii and Pseudomonas aeruginosa: first report from Pakistan. *Revista da Sociedade Brasileira de Medicina Tropical, 52*, e20190237.

Imtiaz, W., Syed, Z., Rafaque, Z., Andrews, S. C., & Dasti, J. I. (2021). Analysis of antibiotic resistance and virulence traits (genetic and phenotypic) in Klebsiella pneumoniae clinical isolates from Pakistan: identification of significant levels of carbapenem and colistin resistance. *Infection and drug resistance*, 227-236.

Inam, S., Ikram, S., Malik, I. Y., Jabeen, K., & Saeed, M. T. (2023). Current Trends In Blood Stream Infections-Pathogens And Their Antimicrobial Susceptibility Pattern. *Pakistan Postgraduate Medical Journal*, *34*(02), 83-87.

Jabeen, F., Khan, Z., Sohail, M., Tahir, A., Tipu, I., & Murtaza Saleem, H. G. (2022). Antibiotic Resistance Pattern Of Acinetobacter Baumannii Isolated From Bacteremia Patients In Pakistan. *Journal of Ayub Medical College Abbottabad-Pakistan*, *34*(1).

Jean, S.-S., Harnod, D., & Hsueh, P.-R. (2022). Global threat of carbapenem-resistant Gram-negative bacteria. *Frontiers in cellular and infection microbiology*, *12*, 236.

Jiang, Y., Ding, Y., Wei, Y., Jian, C., Liu, J., & Zeng, Z. (2022). Carbapenem-resistant Acinetobacter baumannii: A challenge in the intensive care unit. *Frontiers in Microbiology*, *13*, 1045206.

Kern, W., & Rieg, S. (2020). Burden of bacterial bloodstream infection—a brief update on epidemiology and significance of multidrug-resistant pathogens. *Clinical Microbiology and Infection*, *26*(2), 151-157.

Moubareck, C. A., & Halat, D. H. (2020). Insights into Acinetobacter baumannii: a review of microbiological, virulence, and resistance traits in a threatening nosocomial pathogen. *Antibiotics*, *9*(3), 119.

Munir, S., Inam, S., Aslam, A., Aslam, M., Nasir, U., Muddassir, M., . . . Maqbool, T. (2021). Gram negative organisms isolated from blood cultures and their susceptibility pattern in a tertiary care hospital. *Pak J Med Health Sci*, *15*(5), 1074-1079.

Nasiri, M. J., Mirsaeidi, M., Mousavi, S. M. J., Arshadi, M., Fardsanei, F., Deihim, B., . . . Goudarzi, H. (2020). Prevalence and mechanisms of carbapenem resistance in Klebsiella pneumoniae and Escherichia coli: a systematic review and meta-analysis of cross-sectional studies from Iran. *Microbial Drug Resistance*, *26*(12), 1491-1502.

Novović, K., & Jovčić, B. (2023). Colistin resistance in Acinetobacter baumannii: molecular mechanisms and epidemiology. *Antibiotics*, *12*(3), 516.

Papathanakos, G., Andrianopoulos, I., Papathanasiou, A., Priavali, E., Koulenti, D., & Koulouras, V. (2020). Colistin-resistant Acinetobacter baumannii bacteremia: a serious threat for critically ill patients. *Microorganisms*, *8*(2), 287.

Pormohammad, A., Mehdinejadiani, K., Gholizadeh, P., Nasiri, M. J., Mohtavinejad, N., Dadashi, M., . . . Azimi, T. (2020). Global prevalence of colistin resistance in clinical isolates of Acinetobacter baumannii: A systematic review and meta-analysis. *Microbial pathogenesis*, *139*, 103887.

Qin, J., Zou, C., Tao, J., Wei, T., Yan, L., Zhang, Y., & Wang, H. (2022). Carbapenem resistant Pseudomonas aeruginosa infections in elderly patients: antimicrobial resistance profiles, risk factors and impact on clinical outcomes. *Infection and Drug Resistance*, 2301-2314.

Santimaleeworagun, W., Thunyaharn, S., Juntanawiwat, P., Thongnoy, N., Harindhanavudhi, S., Nakeesathit, S., & Teschumroon, S. (2020). The prevalence of colistin-resistant Gram-negative bacteria isolated from hospitalized patients with bacteremia. *Journal of Applied Pharmaceutical Science*, *10*(2), 056-059.

Uzairue, L. I., Rabaan, A. A., Adewumi, F. A., Okolie, O. J., Folorunso, J. B., Bakhrebah, M. A., . . . Alamri, A. A. (2022). Global prevalence of colistin resistance in Klebsiella pneumoniae from bloodstream infection: A systematic review and meta-analysis. *Pathogens*, *11*(10), 1092.

Wu, C., Zheng, L., & Yao, J. (2022). Analysis of risk factors and mortality of patients with carbapenem-resistant Klebsiella pneumoniae infection. *Infection and Drug Resistance*, 2383-2391.

Yusuf, E., van Westreenen, M., Goessens, W., & Croughs, P. (2020). The accuracy of four commercial broth microdilution tests in the determination of the minimum inhibitory concentration of colistin. *Annals of Clinical Microbiology and Antimicrobials*, *19*, 1-8.