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Original Article

Changing Trends of Antimicrobial Resistance in Clinical Isolates Yielded from Lower Respiratory Tract Specimens of ICU Patients-A Two-Year Study

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ABSTRACT

Objective: To determine changing trends in antimicrobial resistance among Gram-negative bacterial isolates yielded in lower respiratory tract specimens in intensive care settings.

Study Design: Cross-sectional study

Place and Duration of Study: Armed Forces Institute of Pathology, Rawalpindi Pakistan, Jul 2018 to Jun 2020.

Methodology: The study was carried out on 819 isolates from lower respiratory tract specimens collected from ICUs of multiple centres all over Pakistan. The antimicrobial susceptibility testing was performed according to performance standards provided by CLSI and EUCAST. Antimicrobial resistance trends were analyzed.

Results: In *Acinetobacter baumannii*, resistance increased against Carbapenems (92% to 97.4%) and Polymyxins (0% to 5.3%). *Pseudomonas aeruginosa* showed increasing resistance, with Imipenem (33.3% to 46.9%), Meropenem (27.3% to 43.6%) and Polymyxin (0% to 3%). In *Klebsiella pneumoniae*, the resistance against Carbapenems rose from 60.5% to 79.2%, for Imipenem 68.4% to 83% for Meropenem. Polymyxin resistance was much higher in *K. pneumoniae* (increasing from 22% to 24.5%). In *Escherichia coli*, resistance increased from 23.5% to 41.7% for Imipenem 35.5% to 50% for Meropenem, and 0% to 8.3% for Polymyxins, whereas Tigecycline showed decreasing resistance trend. Other antimicrobials tested showed increasing resistance as well.

Conclusion: Antimicrobial resistance is emerging so rapidly that the post-antibiotic era is approaching sooner than later. Extensive and up-to-date insight regarding antimicrobial resistance rates and trends against significant pathogens is required to deal with this emerging dilemma.

Keywords: Antimicrobial resistance, Carbapenems, Intensive care, Polymyxins, Tigecycline, Trends.

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INTRODUCTION

Antimicrobial resistance (AMR) is one of the significant challenges currently encountered in healthcare settings.[1] Gram-negative bacteria constitute a significant portion of isolates yielded from lower respiratory tract (LRT) specimens and are mostly found to be highly drug-resistant. Emerging resistance to antimicrobials in healthcare-associated bacterial isolates limits the treatment options available, thus leading to increased morbidity and mortality.[2]

An increase in AMR has led to increased utilization of terminologies such as multidrug-resistant (MDR), extensively drug-resistant (XDR) and pan-drug-resistant (PDR) bacteria. A joint initiative by the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) has provided standardized definitions for these terminologies.[3] MDR is defined as acquired resistance to at least one agent in three or more antimicrobial classes, XDR is defined as resistance to at least one agent in all but two or fewer antimicrobial classes (*i.e.*, bacterial isolates remain susceptible to only one or two classes) and PDR is defined as resistance to all agents in all antimicrobial classes.[4]

Commonly isolated Gram-negative bacterial pathogens in healthcare settings include *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and other Enterobacterales.[5] These isolates mostly turn out to be highly drug-resistant and are thus difficult to treat. This leads to a problematic situation in critical care settings, where these drug-resistant isolates are responsible for high morbidity and mortality.[6]

Antimicrobial resistance (AMR) is an emerging crisis that significantly threatens public health. It leads to ineffective antimicrobial usage, increased mortality, extended hospital stays, and an enormous economic burden. Antimicrobial stewardship programs (ASPs)

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are an essential strategy among the action plans against AMR. ASPs have multiple components, one of which is monitoring and regular reporting of antibiotic usage and resistance patterns.[7] The clinicians must remain well-informed of the antimicrobial resistance patterns and changing trends. This leads to improved patient care and management and helps take necessary precautions to deal with this emerging threat.

METHODOLOGY

The cross-sectional study was conducted at the Armed Forces Institute of Pathology (AFIP), Rawalpindi, over two years (July 2018 to June 2020) after approval by the Institutional Review Board (Cons-MIC-4/READ-IRB/21/214).

Inclusion Criteria: Lower respiratory tract samples from patients of all ages and genders were included.

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

The samples were collected from Intensive Care settings, including Medical, Surgical and Paediatric Intensive Care Units (ICUs), Bone Marrow Transplant Centres, a Urology setup, and a Liver Transplant Unit. This was a time-barred study, and all lower respiratory tract specimens received for culture and sensitivity during the study period were included using a non-probability consecutive sampling technique. A total of 819 isolates from LRT samples, i.e., bronchoalveolar lavage (BAL), bronchial washings, non-directed bronchial lavage (NBL) and sputum samples, were studied.Bacterial isolation and identification were done according to standard microbiological procedures. Antimicrobials tested for each isolate were according to performance standards provided by the Clinical and Laboratory Standards Institute (CLSI) - M100 document and European Committee on Antimicrobial Susceptibility Testing (EUCAST).[8]

The antimicrobial susceptibility testing (AST) was performed according to performance standards provided by CLSI - M100 document. Disk diffusion was performed using Mueller Hinton (MH) agar, and zone diameters were determined for AST, except for Polymyxins for which Colistin agar test and broth microdilution were used to determine minimal inhibitory concentrations (MICs). For Tigecycline AST, breakpoints given according to EUCAST were used.

Statistical Package for Social Sciences (SPSS) version 22.0 was used for the data analysis. Qualitative variables were expressed as frequency & percentages.

RESULTS

Isolates included in the study were the commonly yielded Gram-negative bacteria in Intensive Care Settings, i.e., *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. The most frequently isolated pathogen was *A. baumannii* (n=261, 31.9%), followed by *P. aeruginosa* (n= 256, 31.3%) and *K. pneumoniae* (n=251, 30.6%). The distribution of these isolates over two years is depicted in Table-I.

Table-I: Number of Isolates during two year Study period (n=819)

Duration	Acinetobacter Baumannii	Pseudomonas Aeruginosa	Klebsiella Pneumoniae	Escherichia Coli	Total
Jul to Dec 2018	50	66	76	17	209
Jan to Jun 2019	68	80	50	13	211
Jul to Dec 2019	86	78	72	9	245
Jan to Jun 2020	57	32	53	12	154
Total	261(31.9%)	256(31.3%)	251(30.6%)	51(6.2%)	819

The percentage resistance of the studied Gram-negative bacterial isolates against different antimicrobials is shown in Table-II.

In *Acinetobacter baumannii*, resistance increased against Carbapenems (92% to 97.4%) and Polymyxins (0% to 5.3%). *Pseudomonas aeruginosa* showed increasing resistance, with Imipenem (33.3% to 46.9%), Meropenem (27.3% to 43.6%) and Polymyxin (0% to 3%). In *Klebsiella pneumoniae*, the resistance against Carbapenems rose from 60.5% to 79.2% for Imipenem and 68.4% to 83% for Meropenem. Polymyxin resistance was much higher in *K. pneumoniae* (increasing from 22% to 24.5%). In *Escherichia coli*, resistance increased from 23.5% to 41.7% for Imipenem, 35.5% to 50% for Meropenem, and 0% to 8.3% for Polymyxins, whereas Tigecycline showed decreasing resistance trend. Other antimicrobials tested showed increasing resistance as well.

DISCUSSION

In our setup, we encountered *A. baumannii*, *P. aeruginosa*, *K. pneumoniae* and *E. coli* as the most commonly isolated bacteria from LRT specimens. All these isolates turned out to be highly drug-resistant, with resistance increasing gradually for most of the antimicrobials over a period of two years.

Table-II: Antimicrobial Percentage Resistance during the two year Study period (n=819)

Acinetobacter baumannii (Percentage Resistant)

Duration	CIP (%)	IMI (%)	MEM (%)	GM (%)	AK (%)	TZP (%)	FEP (%)	CRO (%)	DOX (%)	MIN (%)	COT (%)	PB/Col (%)
Jul to Dec 2018 (n=50)	47(94.0)	46(92.0)	46(92.0)	35(70.0)	42(84.0)	46(92.0)	48(96.0)	48(96.0)	15(30.0)	11(22.0)	46(92.0)	0(0.0)
Jan to Jun 2019 (n=68)	63(92.6)	64(94.1)	64 (94.1)	56(82.4)	62(91.2)	64(94.1)	65(95.6)	67(98.6)	22 (32.4)	16 (23.5)	60 (88.2)	3(2.9)
Jul to Dec 2019 (n=86)	79(91.9)	81(94.2)	82 (95.3)	74(86.0)	78(90.7)	82(95.3)	79(91.9)	85 (98.8)	24 (27.9)	24 (27.9)	77 (89.5)	0.0(0.0)
Jan to Jun 2020 (n=57)	56(98.2)	55(96.5)	56(98.2)	53(93.0)	54(94.7)	55(96.5)	56(98.2)	56(98.2)	19(33.3)	17(29.8)	55(96.5)	3(5.3)

Pseudomonas aeruginosa (Percentage resistant)

Duration	CIP (%)	IMI (%)	MEM (%)	GM (%)	AK (%)	TZP (%)	FEP (%)	CAZ (%)	AZT (%)	LEV (%)	PB/Col (%)
Jul to Dec 2018 (n=66)	27(40.9)	22(33.3)	18(27.3)	19(28.9)	16(24.2)	17(25.7)	17(25.7)	17(25.7)	21(31.8)	22(33.3)	0.0(0.0)
Jan to Jun 2019 (n=80)	33(41.3)	29(36.3)	31(38.6)	27(33.8)	22(27.5)	30(37.5)	24(30.0)	34(42.5)	44(55.0)	34(42.5)	0.0(0.0)
Jul to Dec 2019 (n=78)	36(46.2)	31(39.7)	32(41.0)	36(46.2)	30(38.5)	23(29.4)	28(35.9)	35(44.9)	33(42.3)	31(39.7)	1(1.2)
Jan to Jun 2020 (n=32)	18(56.3)	15(46.9)	14(43.6)	15(46.9)	13(40.6)	10(31.3)	12(37.5)	12(37.5)	12(37.5)	15(46.9)	1(3.0)

Klebsiella pneumoniae (Percentage resistant)

Duration	CIP (%)	AMC (%)	COT (%)	IMI (%)	MEM (%)	GM (%)	AK (%)	TZP (%)	FEP (%)	CRO (%)	DOX (%)	TGC (%)	PB/Col (%)
Jul to Dec 2018 (n=76)	65(85.5)	72(94.7)	55 (72.4)	46(60.5)	52(68.4)	54(71.0)	52 (68.4)	69(90.7)	69(90.7)	69(90.7)	37(48.7)	19(25.0)	17(22.4)
Jan to Jun 2019 (n=50)	40(80.0)	46(92.0)	38(76.0)	33(66.0)	35(70.0)	36(72.0)	33(66.0)	45(90.0)	46(92.0)	46(92.0)	27(54.0)	9(18.0)	11(22.0)
Jul to Dec 2019 (n=72)	68(94.4)	71(98.6)	51(70.8)	51(70.8)	72(80.6)	63(87.5)	56(77.7)	64(88.9)	68(94.4)	68(94.4)	36(50.0)	12(16.6)	17(23.6)
Jan to Jun 2020 (n=53)	48(90.6)	50(94.3)	43(81.1)	42(79.2)	44(83.0)	47(88.7)	41(77.3)	47(88.7)	50(94.3)	49(92.5)	27(50.9)	1(1.9)	13(24.5)

Escherichia coli (Percentage resistant)

Duration	AMP (%)	CIP (%)	AMC (%)	COT (%)	IMI (%)	MEM (%)	GM (%)	AK (%)	TZP (%)	FEP (%)	CRO (%)	DOX (%)	TGC (%)	PB/Col (%)
Jul to Dec 2018 (n=17)	17(100)	14(82.4)	16(94.1)	11(64.7)	4(23.5)	6(35.3)	8(47.1)	6(35.3)	16(94.1)	15(88.2)	16(94.1)	11(64.7)	1(5.9)	0.0 (0.0)
Jan to Jun 2019 (n=13)	12(92.3)	12(92.3)	12(92.3)	11(84.6)	3(23.1)	5(38.4)	9(69.2)	5(38.5)	11(84.6)	12(92.3)	12(92.3)	10(76.9)	0.0(0.0)	0.0 (0.0)
Jul to Dec 2019 (n=9)	9(100)	8(88.9)	9(100)	7(77.8)	3(33.3)	4(44.4)	6(66.7)	4(44.4)	8(88.9)	8(88.9)	8(88.9)	9(100)	0.0(0.0)	0.0 (0.0)
Jan to Jun 2020 (n=12)	12(100)	10(83.3)	11(91.7)	11(91.7)	5(41.7)	6(50.0)	6(50.0)	5(41.7)	11(91.7)	11(91.7)	11(91.7)	8(66.7)	0.0(0.0)	1 (8.3)

[CIP-Ciprofloxacin, IMI-Imipenem, MEM-Meropenem, GM-Gentamicin, AK-Amikacin, TZP-Piperacillin/Tazobactam, FEP-Cefepime, CRO-Ceftriaxone, DOX-Doxycycline, MIN-Minocycline, COT-Trimethoprim/Sulfamethoxazole, PB-Polymyxin B, Col-Colistin, CAZ-Ceftazidime, AZT-Aztreonam, LEV-Levofloxacin, AMC-Amoxicillin/ Clavulanate, TGC-Tigecycline, AMP-Ampicillin]

Our study shows that *A. baumannii* has increasingly acquired resistance to the significant antibiotic classes available over the two-year study period. The resistance against Carbapenems was most noteworthy, which increased from 92% to 97.4% during this alarming study. In our study, resistance to Polymyxins, including Colistin, was not observed among *A. baumannii* at the beginning of the two years. However, 5.3% of isolates were polymyxin resistant at the end of this period. This trend poses a serious threat to healthcare settings, making treating this perilous bug even more complex and turning it into an even deadlier threat than before.[9,10]

The increasing prevalence of highly drug-resistant *Pseudomonas aeruginosa* strains is another severe problem in selecting appropriate antimicrobial treatment resulting in significant morbidity and mortality.[11,12] This emerging threat is due to the capability of this pathogen to develop resistance to almost all available antibiotics.[13]

Polymyxins are an important therapeutic option in carbapenem-resistant *p. aeruginosa* isolates since these isolates are mostly MDR or XDR. Nonetheless, Polymyxin resistance is also on the rise during the two-year period, gradually rising from 0 to 3%.[14]

Other major antimicrobials tested for *P. aeruginosa* (including Aminoglycosides, Fluoroquinolones and β-lactam antibiotics) showed increasing AMR trends over the two years.[15]

Polymyxins are the mainstay of therapy for Carbapenem-resistant *K. pneumoniae* isolates in many clinical settings. However, in our study, we found a much higher rate of Polymyxin resistance in *K. pneumoniae* as compared to other bacterial isolates included in the study. The resistance rate was 22% at the start and 24.5% at the end of the study, showing a high percentage of resistance even at the start, with a gradual increase over the two years. This is alarming and must be addressed as a serious threat to patient care and management.[16]

Other than Polymyxins, Doxycycline and Tigecycline have shown better results against *K. pneumoniae*, and these drugs were the only option for XDR isolates with polymyxin resistance in many cases.[17]

MDR *Escherichia coli* has become a significant public health concern in many countries across the globe. Again, this presents a significant challenge in treating HAIs, causing treatment failures with consequent huge health burdens.[18] In our study, *E. coli* was found to be MDR and XDR in most cases. However, the resistance rates were comparatively lower than other isolates, particularly *K. pneumoniae*. The resistance rates were nonetheless increasing over the two years. Resistance against carbapenems increased gradually during the study, 23.5% to 41.7% for imipenem and 35.5% to 50% for meropenem.

Tigecycline was the only drug that showed decreasing resistance trends for *E. coli* and *K. pneumoniae*, making it an essential option for managing MDR and XDR pathogens showing resistance to carbapenems and polymyxins.[19]

Thus, the trends observed during the study present quite alarming facts regarding the use of antimicrobials. Resistance rates are increasing rapidly against most antimicrobials, including carbapenems and polymyxins, which are currently considered among the drugs of last resort against highly drug-resistant bugs. Growing AMR is a nightmare for healthcare personnel, making the management of infections complicated with each passing day. Large-scale multicenter studies are required to assess and evaluate the AMR trends to formulate strategies for region and hospital-based antibiotic policies. Moreover, it is the need of the hour to observe antimicrobial stewardship programs in all healthcare settings to curb the menace of AMR.

CONCLUSION

The overall state of AMR trends observed during the two-year study period shows that AMR is emerging so rapidly that the post-antibiotic era is approaching sooner than later. There is an immense requirement for extensive and up-to-date insight regarding the AMR rates and trends against the major pathogens. At the same time, antimicrobial stewardship programs must be implemented religiously and vigorously to curb this threat before it is too late.

Conflict of Interest: None.

Authors Contribution

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

MS: & IAM: Study design, drafting the manuscript, concept, approval of the final version to be published.

AI: & WH: Critical review, data acquisition, drafting the manuscript, approval of the final version to be published.

UK: & AHC: Data acquisition, data analysis, data interpretation, 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.

REFERENCES

1. Ramsamy Y, Essack SY, Sartorius B, Patel M, Mlisana KP. Antibiotic resistance trends of ESKAPE pathogens in Kwazulu-Natal, South Africa: A five-year retrospective analysis. Afr J Lab Med 2018 ; 7(2): 887. https://doi.org/10.4102/ajlm.v7i2.887.
2. Hay SI, Rao PC, Dolecek C, Day NPJ. Measuring and mapping the global burden of antimicrobial resistance. BMC Med 2018; 16(1): 78-80. https://doi.org/ 10.1186/ s12916-018-1073-z
3. Fox-Lewis A, Takata J, Miliya T, Lubell Y, Soeng S, Sar P, et al. Antimicrobial resistance in invasive bacterial infections in hospitalized children, Cambodia, 2007–2016. Emerg Infect Dis 2018; 24(5): 841-851. https://doi.org/10.3201%2Feid2405.171830
4. Kengkla K, Kongpakwattana K, Saokaew S, Apisarnthanarak A. Comparative efficacy and safety of treatment options for MDR and XDR Acinetobacter baumannii infections: a systematic review and network meta-analysis. J Antimicrob Chemother 2018; 73(1): 22-32. https://doi.org/10.1093/jac/dkx368

5. Moghnieh RA, Kanafani ZA, Tabaja HZ, Sharara SL, Awad LS, Kanj SS, et al. Epidemiology of common resistant bacterial pathogens in the countries of the Arab League. Lancet Infect Dis 2018; 18(12): e379-394. https://doi.org/10.1016/s0001473-3099(18)00030414-6
6. McCann E, Srinivasan A, DeRyke CA, Ye G, DePestel DD, Murray J, et al. Carbapenem-Nonsusceptible Gram-Negative Pathogens in ICU and Non-ICU Settings in US Hospitals in 2017: A Multicenter Study. Open Forum Infect Dis 2018; 5(10): ofy241. https://doi.org/10.1093%2Fofid%2Fofy241
7. Gillespie D, Francis NA, Carrol ED, Thomas-Jones E, Butler CC, Hood K, et al. Use of co-primary outcomes for trials of antimicrobial stewardship interventions. Lancet Infect Dis 2018; 18(6): 595-597. https://doi.org/10.1016/s1473-3099(18)30289-5
8. Watkins RR, Van Duin D. Current trends in the treatment of pneumonia due to multidrug-resistant Gram-negative bacteria. F1000Res 2019; 8(1): F1000 https://doi.org/10.12688%2Ff-1000research.16517.2
9. Maragakis LL, Perl TM. Acinetobacter baumannii: epidemiology, antimicrobial resistance, and treatment options. Clin Infect Dis 2008 ; 46(8): 1254-1263. https://doi.org/10.1086/529198
10. Gao L, Lyu Y, Li Y. Trends in drug resistance of Acinetobacter baumannii over a 10-year period: Nationwide data from the China surveillance of antimicrobial resistance program. Chin Med J 2017 ; 130(6): 659-664. https://doi.org/10.4103%2F0366-6999.201601
11. Isler B, Doi Y, Bonomo RA, Paterson DL. New treatment options against carbapenem-resistant Acinetobacter baumannii infections. Antimicrob Agents Chemother 2019; 63(1): e01110-01118. https://doi.org/10.1128/aac.01110-18
12. Falagas ME, Vardakas KZ, Kapaskelis A, Triarides NA, Roussos NS. Tetracyclines for multidrug-resistant Acinetobacter baumannii infections. Int J Antimicrob Agents 2015; 45(5): 455-460. https://doi.org/10.1016/j.ijantimicag.2014.12.031
13. Fraile-Ribot PA, Cabot G, Mulet X, Periañez L. Mechanisms leading to in vivo ceftolozane/tazobactam resistance development during the treatment of infections caused by MDR Pseudomonas aeru-ginosa. J Antimicrob Chemother 2018; 73(3): 658-663. https:// doi.org/10.1093/jac/dkx424
14. Monogue ML, Nicolau DP. Antibacterial activity of ceftolozane/tazobactam alone and in combination with other antimicrobial agents against MDR Pseudomonas aeruginosa. J Antimicrob Chemother 2018; 73(4): 942-952. https://doi.org/10.1093/jac/dkx483
15. Sousa Dominguez A, Perez-Rodríguez MT, Nodar A, Martinez-Lamas L, Perez-Landeiro A, Crespo Casal M, et al. Successful treatment of MDR Pseudomonas aeruginosa skin and soft-tissue infection with ceftolozane/tazobactam. J Antimicrob Chemother 2017; 72(4): 1262-1263. https://doi.org/10.1093/jac/dkw526
16. Labarca JA, Salles MJ, Seas C. Carbapenem resistance in Pseudomonas aeruginosa and Acinetobacter baumannii in the nosocomial setting in Latin America. Crit Rev Microbiol 2016; 42(2): 276-292. https://doi.org/10.3109/1040841x.2014.940494
17. Yin S, Chen P, You B, Zhang Y, Jiang B, Huang G, et al. Molecular typing and carbapenem resistance mechanisms of Pseudomonas aeruginosa isolated from a Chinese burn center from 2011 to 2016. Front Microbiol 2018; 9(1): 1135. https:// doi.org /10.3389%2Ffmicb.2018.01135
18. Arumugam SN, Rudraradhya AC, Sadagopan S, Sukumaran S, Sambasivam G, Ramesh N, et al. Analysis of susceptibility patterns of pseudomonas aeruginosa and isolation, characterization of lytic bacteriophages targeting multi drug resistant pseudomonas aeruginosa. Biomed Pharmacol J 2018; 11(2): 1105-1117. http://dx.doi.org/10.13005/bpj/1471
19. Both A, Büttner H, Huang J, Perbandt M, Belmar Campos C. Emergence of ceftazidime/avibactam non-susceptibility in an MDR Klebsiella pneumoniae isolate. J Antimicrob Chemother 2017; 72(9): 2483-2488. https://doi.org/ 10.1093/jac/dkx179.