doi.org/10.36721/PJPS.2021.34.5.REG.1737-1741.1

Evaluation of 16S rRNA methyltransferase gene in aminoglycosides resistant isolates of cancer patients

Saba Ghafoor, Basit Zeshan*, Madiha Khan and Saba Kabir*
Department of Microbiology, Faculty of Life Sciences University of the Central Punjab, Lahore, Pakistan

Abstract: Aminoglycosides are used in empiric treatment of critically ill patients. Efficacy of aminoglycoside has been reduced due to dissemination of resistance. The aim of this study was to evaluate aminoglycoside resistance in cancer patients with pneumoniae. A total of 150 Bronchoalveolar lavage and Bronchial washing samples were collected from cancer patients. The samples were identified with standard microbiological procedures. Phenotypic susceptibility pattern of the isolates was determined against various groups of antibiotics such as Penicillins, Cephalosporins, Carbapenems, Monobactams, Aminoglycosides, Tetracyclins, Glycopeptides and Sulphonamides. The isolates with phenotypic resistant to aminoglycosides were further evaluated for the presence of *armA* gene. The strains of E. *coli* (12.5%), *S. aureus* (15.6%), *Streptococcus* (15.6%), *Pseudomonas* (18.7%) and *K. pneumoniae* (37.5%) were isolated. The phenotypic resistance profile showed highest resistance against aminoglycosides (Tobramycin, 53.1% Gentamicin and 50% Amikacin) followed by cephalosporins and sulfonamides group. The *armA* gene was detected in aminoglycoside resistant isolates. The overall genotypic resistance was evaluated as 21.8%. The *armA* gene was found in *K.pneumoniae* 23.5%, *Pseudomonas* 11.8% (4/24) and *E. coli* 5.9%. High level resistance to aminoglycosides raises therapeutic concern to health care professionals. These findings highlight the importance of effective monitoring and surveillance to the use of broad-spectrum antibiotics.

Keywords: Aminoglycosides resistance, *armA* gene, 16S rRNA methyltransferase gene, immunocompromised patients.

INTRODUCTION

Pneumonia is the primary cause of death in children in developing countries accounting for total death rate of 15% under 5 years of age (WHO) and second important cause of death in Pakistan (Fatemah *et al.,* 2019). Pneumonia is frequently caused by bacteria including S*treptococcus pneumoniae (S.pneumoniae), Klebsiella pneumoniae (K. pneumoniae), Haemophilus influenzae (H. influenzae), Pseudomonas aeruginosa (P. aeruginosa)* and *Staphylococcus aureus (S. aureus)* (Sattar and Sharma, 2020). Factors associated with increased risk of pneumonia includes immunodeficiency, chronic kidney diseases, liver diseases, asthma, obstructive pulmonary diseases, smoking and old age (Nair and Niederman 2011 (Heo *et al*., 2020). Bacterial pneumonia causes severe infections in cancerous patients (Wong and Evans 2017). The immune suppression in cancer patients lead to increased risk of pneumonia. Antibiotics are commonly used to treat community acquired bacterial pneumonia. The burden of antibiotic resistance is increasing worldwide (Ho and Ip, 2019). Aminoglycoside are broad spectrum antibiotics that inhibit protein synthesis in bacteria (Shen *et al*., 2020) by binding to A site of 16S rRNA or blocking elongation step (Shen *et al*., 2020). Resistance to aminoglycoside develop through intrinsic and acquired mechanism such as enzymatic drug modification, intracellular antibiotic accumulation, efflux pump or acquired mutations (Tsodikova *et al*., 2016). The 16S rRNA methyl transferases (RMTase) are emerged as a new mechanism of resistance against majority of clinically important aminoglycosides (Galimand *et al*., 2003). The *armA* is the most frequently occurring 16S-RMTase gene along with *rmtB*. It is the most frequently found in the Enterobacteriaceae, *K. pneumoniae, Salmonella enterica* and *Shigella flexneri* (Galimand *et al*., 2005; (Lioy *et al*., 2014). Keeping in view the significance of aminoglycoside resistance in bacterial pneumonia, the current study was designed to determine the prevalence of *armA* gene in our clinical isolates.

MATERIALS AND METHODS

Isolation and identification of bacterial species

A total of 150 bronchoalveolar lavage and bronchoalveolar washings samples were collected from cancer patients. Samples were cultured on Blood agar, MacConkey agar and chocolate agar. The identification of bacterial isolates was done based on macroscopic and microscopic characteristics and confirmed by biochemical testing and analytical profile index (API 20E) (Bergey *et al*., 1984).

Antimicrobial susceptibility assay

Biochemically confirmed isolates were further processed for antibiotic susceptibility pattern by Kirby Bauer disk diffusion method (CLSI, 2017). The antibiotic used for this study were Imipenem (IPM), Meropenem (MEM),

---

**Corresponding author:* e-mails: dr.basitzeshan@ucp.edu.pk; saba.kabir@ucp.edu.pk

Aztreonam (ATM), Amoxicillin (AX), Oxacillin (OX), Ampicillin (AM), Amikacin (AK), Gentamicin (CN), Tobramycin (TOB), Cephradine (CE), Cefepime (FEP), Cefotaxime (CTX), Cefixime (CFM), Cefoperazone (CES), Ceftriaxone (CRO), Vancomycin (VA), Teicoplanin (TEC), Doxycycline (DO) and Trimethoprim (TMP).

Molecular characterization

For molecular characterization, the isolates that were phenotypically resistant to aminoglycoside, were proceeded for the screening of *armA gene.* The DNA was extracted using commercially available Genomic DNA extraction kit (Gene All™). Polymerase Chain Reaction (PCR) was done using already reported *armA* gene primers F:5'ATTTTAGATTTTGGTTGTGGC3'and R: 5'ATCTC AGCTCTATCAATATCG3') of all selected isolates (Fatimah *et al.,*2019).

STATISTICAL ANALYSIS

SPSS version 20 was used for statistical analysis.

RESULTS

Demographic data analysis

A total of 150 samples of Bronchoalveolar lavage (BAL) (n=120) and Bronchial washings (n=30) were collected from cancer patients. The distribution of samples according to the age and gender of the patient is given in table 1. A total 94 samples were collected from male patients mostly from age group 51-60 years. While 56 samples were collected from female patients with highest frequency at same age group.

Bacterial isolation and antimicrobial susceptibility pattern

A total 64 samples were positive for bacterial growth and identified using standard procedures. Various pathogens isolated from these samples were *E. coli* 12.5% (8/64), *S. aureus* 15.6% (10/64), *Streptococcus* 15.6% (10/64), *Pseudomonas* 18.7% (12/64) and *K. pneumoniae* 37.5% (24/64).

Antibiogram of these isolates showed the resistance to various class of antibiotics. The highest resistance was observed against aminoglycoside group. Out of 64 isolates 34 isolates showed resistance to at least one aminoglycoside. The number of pathogens resistant to aminoglycoside drug was *K. pneumoniae* 15, *Pseudomonas* 9, *E. coli* 5, *S. aureus* 3 and *Streptococcus* 2. The highest resistance rate was found as 56.2% in Tobramicin, 53.1% Gentamicin and 50% Amikacin. The resistance to other drugs was as follows: Amoxicillin 37.5%, Oxacillin 21.8%, Ampicillin 31.2%, Cephradine (CE) 50%, Cefepime 28.1%, Cefotaxime 43.7%, Cefixime 40.6%, Cefoperazone 15.6%, Ceftriaxone 43.7%, Imipenem 15.6%, Meropenem 18.7%, Azetreonam 46.8%, Doxycyclin 43.7%, Vancomycin 37.5%, Teicoplanin 18.7% and Trimethoprim 40.6% (table 2).

Detection of armA gene

Phenotypically resistant strains (n=34) were subjected to amplification of *armA* gene. A total of 14 isolates were positive for *armA* gene. The *armA* gene was most prevalent in *K. pneumoniae* 8(23.5%). Whereas the rate of *armA* gene in *Pseudomonas* was 4(11.8%) and *E. coli* 2(5.9%) (fig. 1).

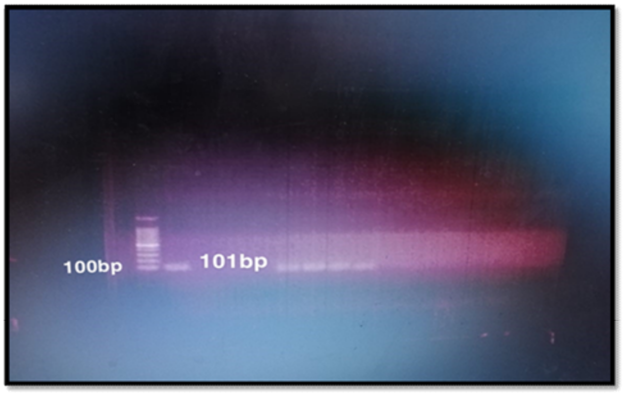

Fig. 1: DNA band of *armA* gene with amplicon size of 101 bp

DISCUSSION

Pneumonia is the one of the leading causes of morbidity and mortality in developing countries. Different factors such as immunodeficiency are associated with increased risk of pneumonia. In the current study, the association of bacterial pneumonia with cancer patients and their antibiotic resistance was studied. The organisms were isolated from BAL and bronchial washings samples. A total of 64 bacterial species were isolated from 150 samples and identified as *E. coli* (8 strains), *Klebsiella pneumonia* (24 strains), *Pseudomonas* (12 strains), *S.aureus* (10 strains) and *Streptococcus spp.* (10 strains). The same isolates were reported in the study of USA (Helio *et al.,* 2014). Another study also reported isolates of *S. aureus, Klebsiella, E. coli* and *Enterobacter* spp. from nosocomial and hospital acquired pneumonia (Tanaseanu *et al*., 2008). In our study, *K. pneumoniae* was found as the most important microorganism associated with pneumonia in cancer patients. The organisms are nosocomial pathogen which can colonize the patients living in hospital environment. A study in Belgium also in line with our results where *K. pneumoniae* was most prevalent organisms in pneumonia patients (Bogaerts *et al*., 2007).

In present study, *P. aeruginosa* was the second most prevalent pathogen among our isolates. *P. aeruginosa* is primarily an opportunistic pathogen that causes infections

Table 1: Demographic history of patients

Age group (years)	**Gender**		**Total**
	Male	Female	
1 -10	2	2	4
11-20	6	2	8
21-30	8	10	18
31-40	16	8	24
41-50	18	10	28
51- 60	20	12	32
61-70	14	8	22
71-80	10	4	14
Total	94	56	150

Table 2: Resistance pattern of isolates (n=64) against different antibiotics

Antibiotic	Sensitive	Resistant
Amoxicillin (AX)	40(62.5%)	24(37.5%)
Oxacillin (OX)	50(78.1%)	14(21.8%)
Ampicillin (AM)	44(68.7%)	20(31.2%)
Cephradine (CE)	32(50%)	32(50%)
Cefepime (FEP)	46(71.8%)	18(28.1%)
Cefotaxime (CTX)	36(56.2%)	28(43.7%)
Cefixime (CFM)	38(59.3%)	26(40.6%)
Cefoperazone (CES)	54(84.3%)	10(15.6%)
Ceftriaxone (CRO)	36(56.2%)	28(43.7%)
Imipenem (IPM)	54(84.3%)	10(15.6%)
Meropenem (MEM)	52(81.2%)	12(18.7%)
Azetreonam (ATM)	34(53.1%)	30(46.8%)
Amikacin (AK)	32(50%)	32(50%)
Gentamicin (CN)	30(46.8%)	34(53.1%)
Tobramicin (TOB)	28(43.8%)	36(56.2%)
Doxycyclin (DO)	36(56.2%)	28(43.7%)
Vancomycin (VA)	40 (62.5%)	24 (37.5%)
Teicoplanin (TEC)	52 (81.2%)	12 (18.7%)
Trimethoprim (TMP)	38 (59.3%)	26 (40.6%)

in hospitalized patients. Its ability to grow in aqueous solutions could result in contamination of respiratory therapy devices. It can also colonize the upper respiratory tract of hospitalized patients by formation of biofilms. In the previous study *P. aeruginosa* was reported as the most prevalent pathogen that cause pneumonia in the patients (Helio *et al*., 2014). *E. coli, S.aureus* and *Streptococcus spp.* were also found as the causative agents of pneumoniae. A study in USA reported *S. aureus* and *Streptococcus* spp. was the leading cause of pneumonia. Among Gram negative organisms, the increased number of *E. coli* were isolated (Heilo *et al.,* 2014).

Aminoglycosides are the antibiotics with potential to use in life threatening infections. Resistance to these antibiotics is the alarming condition. Incidence of aminoglycoside resistance has changed over time (Miro *et al*., 2013). In current study, the incidence of aminoglycoside resistance in patients of pneumonia was studied. The phenotypic resistance to aminoglycosides (amikacin, gentamicin, and tobramycin) was determined as 53.1% (34/64). This possible cause for the high number of resistances could be self-medication and unjudicial use of the medicine. Based on these results, amikacin had least resistance rate about 50% and tobramycin had highest level of resistance 56.2%. These results were similar with the result of Spain in which amikacin had the least resistance level (Miro *et al*., 2013). The results were also comparable with the study in Iran, they showed the highest level of resistance of Streptomycin and least resistance in amikacin (Fatemah *et al*., 2019).

High level resistance to aminoglycoside is encoded by 16S rRNA methylase gene (Galimand *et al*., 2003; Yokoyama *et al.,* 2003). The 16S rRNA methylase gene have been found in the *P. aeruginosa* and Enterobacteriaceae (YU *et al*., 2009). Methylation of the 16S rRNA presents high level resistance to most clinically useful aminoglycosides by inhibiting their access to the site of action. The methylase gene found in DNA and other genetic material like transposon and propagated among Gram-negative bacteria through horizontal gene transfer (Yu *et al.,* 2009). The recent research data indicate a high level of resistance in *E. coli* and Klebsiella over the years (Tawfiq and Abed 2009; Saeed *et al.,* 2010). In our study no genotypic resistance is found in Gram positive organisms against *armA* gene. However, Enterobacteriaceae are more resistant to aminoglycosides as compared to other groups of antibiotics like penicillin, cephalosporin, carbapenem, monobactam, glycopeptides and tetracyclines.

Among the 16S rRNA methylase gene *armA* is most dominant. The *armA* found in many species of Gram-negative bacteria in China, Japan, Korea and Taiwan (Yu *et al.,* 2009). In this study, although, 34 out of 64 isolates were phenotypic resistant to aminoglycosides but only 14 isolates among resistant organisms were positive for *armA* gene. The discordance between phenotype and genotype results may belong to other resistance mechanism like alteration in permeability and efflux pump (Ghotaslou *et al.,* 2017). The overall genotypic resistance was evaluated as 21.8% (14/64). We characterized 8(23.5%) isolates of *Klebsiella*, 4(11.8%) isolates of *Pseudomonas* and 2(5.9%) isolates of *E. coli* as harboring *arm A* resistance gene. The findings are comparable with the study where 28.9% isolates of *K. pneumoniae* were resistant to aminoglycoside methylase gene (AL-Muqdadi and AL-Saadi *et al.,* 2020). The rate of the *armA* gene resistance in other regions was as follows: China (42.3%) (Liao *et al.,* 2020), France (1.3%) (Bercot *et al.,* 2008), Belgium (0.12%) (Bogaerts *et al.,* 2007).

CONCLUSION

Aminoglycosides resistance have major influence on the treatment of serious infections. Various Gram positive and Gram-negative strains were found associated with pneumonia in cancer patients. A developing trend of gram-negative bacteria with increasing rate of resistance to aminoglycosides is a serious clinical problem. The dissemination of 16sRNA methyltransferase gene contributed to resistance of these broad-spectrum antibacterial drugs. Therefore, comprehensive studies for molecular detection of these genes should be conducted to control spread of the resistance.

ACKNOWLEDGMENTS

The authors are grateful to the University of central Punjab Lahore, for providing platform to conduct this research.

REFERENCES

Aatekah O, Shiyam ST and Shazia S (2010). Incidence of pneumonia, bacteremia and invasive pneumococcal disease in Pakistani children. *Trop. Med. Int. Health*, **15**(9): 1029-1036.

Aliberti S (2009). The pneumonia severity index and the CRB-65 in cancer patients with community-acquired pneumonia. *Int. J. Tuberc. Lung Dis*., **13**(12): 15.

Al Sheikh YA, Marie MAM and John J (2014). Prevalence of 16S rRNA methylase genes among β-lactamase-producing Enterobacteriaceae clinical isolates in Saudi Arabia. *Libyan J. Med*., **9**(1): 24432

Al-Muqdadi BMJ and AL-Saadi BQH (2020). Detection of arma gene, kpc enzyme and molecular typing of *K. pneumoniae* clinical isolate from public hospitals in baghdad city, Iraq. *Biochem. Cell. Arch*., **20**: 1.

Al-Tawfiq JA and Abed MS (2009). Prevalence and antimicrobial resistance of health care associated bloodstream infections at a general hospital in Saudi Arabia. *Saudi Med J.,* **30**(9): 1213-1218.

Ashby B and Turkington C (2007). The encyclopedia of infectious diseases New York: Facts on File. ISBN 978-0-8160-6397-0. 242

Bercot B, Poirel L and Nordmann P (2008) Plasmid mediated 16S rRNA methylases among extended-spectrum b-lactamase-producing Enterobacteriaceae isolates. *Antimicrob. Agents Chemother*., **52**(12): 4526-4527.

Bergey DH, Krieg NR and Holt JG (1984) Bergey's manual of systematic bacteriology. Baltimore, MD: Williams & Wilkins.

Black RE, Cousens S and Johnson HL (2010). Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet*., **375** (9730): 1969-1987.

Bogaerts P, Galimand M and Bauraing C (2007). Emergence of ArmA and RmtB aminoglycoside resistance 16S rRNA methylases in Belgium. *J. Antimicrob. Chemother*., **59**(3): 459-464.

CLSI. Performance Standards for Antimicrobial Susceptibility Testing. (2017). CLSI supplement M100. Clinical and Laboratory Standards Institute.

Doi Y, Wachino J and Arakawa Y (2008). Nomenclature of plasmid-mediated 16S rRNA methylases responsible for panaminoglycoside resistance. *Antimicrob. Agents Chemother*., **52**(6): 2287-2288.

Eddy and Orin (2005). Community-Acquired Pneumonia: From common pathogens to emerging resistance. *Emerg. Med. Pract*., **7**(12): 1-22

Fatemeh YS, Yalda MA and Reza G (2019). High-Level Resistance to Aminoglycosides due to 16S rRNA Methylation in Enterobacteriaceae Isolates. *Microb. Drug. Res*., **25**(9): 1261-1265

Galimand M, Sabtcheva S, Courvalin P and Lambert T (2005). Worldwide disseminated armA aminoglycoside resistance methylase gene is borne by composite transposon Tn1548. *Antimicrob. Agents Chemother*., **49**(7): 2949-2953.

Galimand M, Sabtcheva S and Kantardjiev T (2003). The *armA* aminoglycoside resistance methylase is disseminated in Enterobacteriaceae by an IncL/M plasmid mediating CTX-M3 β-lactamase. In: Proceedings of the 43th Annual Interscience Conference on *Antimicrob. Agents Chemother*. Pp.14-17.

Garneau-Tsodikova S and Kristin JL (2016). Mechanisms of resistance to aminoglycoside antibiotics: Overview and perspectives. *Med. Chem. Comm*., **7**(1): 11-27.

Galimand M, Courvalin P and Lambert TJA (2003). Plasmid-mediated high-level resistance to aminoglycosides in Enterobacteriaceae due to 16S rRNA methylation. *Antimicrob. Agents Chemother.,* **47**(8): 2565-2571

Ghotaslou RF, Yeganeh Sefidan MT and Akhi M (2017). Dissemination of genes encoding aminoglycoside-modifying enzymes and armA among Enterobacteriaceae isolates in Northwest Iran. *Microb Drug Res*., **23**(7): 826-832.

Gonzalez-Zorn BA, Catalan JA and Escudero L (2005). Genetic basis for dissemination of armA. *J. Antimicrob. Chemother*., **56**(3): 583-585.

Heilo CM and Payasi A (2014). Resistance patterns and prevalence of the aminoglycoside modifying enzymes in clinical isolates of gram negative pathogens. *Global J. Pharmacol*., **8**: 73-79.

Heo JW, Yeo CD and Park CK (2020). Smoking is associated with pneumonia development in lung cancer patients. *J. BMC Pulm. Med.,* **20**(1): 1-8.

Ho J and Ip M (2019) Antibiotic-resistant community-acquired bacterial pneumonia. *Infect. Dis. Clin.*, **33**(4): 1087-1103.

Hoheisel G (2003). Nosocomial pneumonias in haematological malignancies in the medical intensive care unit. *Pneumologie*., **57**(2): 73-77.

Liao W, Wang LD and Li D (2020). High Prevalence of 16S rRNA methylase genes among carbapenem-resistant hypervirulent *Klebsiella pneumoniae* isolates in a Chinese Tertiary Hospital. *Microb. Drug Res*.,

Lioy VS, Goussard S and Guerineau V (2014). Aminoglycoside resistance 16S rRNA methyl transferases block endogenous methylation, affect translation efficiency and fitness of the host. *Rna.*, **20**(3): 382-391.

Miro E, Grunbaum F and Gomez L (2013). Characterization of aminoglycoside-modifying enzymes in Enterobacteriaceae clinical strains and characterization of the plasmids implicated in their diffusion. *Microb. Drug Res*., **19**(2): 94-99.

Nair GB and Niederman MS (2011). Community-acquired pneumonia: An unfinished battle. *Med. Clin. N. Am*., **95**(6): 1143-1161

Sattar SBA and Sharma S (2020) Bacterial pneumonia. J. StatPearls. https://www.ncbi.nlm.nih.gov/books/NBK513321/

Saeed NK, Kambal AM and El-Khizzi NA (2010). Antimicrobial resistant bacteria in a general intensive care unit in Saudi Arabia. *Saudi Med. J*., **31**(12): 1341–9

Shen X, Liu L and Yu J (2020). High Prevalence of 16S rRNA Methyltransferase Genes in Carbapenem-Resistant *Klebsiella pneumoniae* Clinical Isolates Associated with Bloodstream Infections in 11 Chinese Teaching Hospitals. *Infect. Drug Resist.*, **13**: 2189.

Tanaseanu C, Bergallo C and Teglia O (2008). Integrated results of 2 phase 3 studies comparing tigecycline and levofloxacin in community-acquired pneumonia. *Diagn. Microbiol. Infect. Dis.*, **61**(3): 329-338.

Yokoyama K, Doi K and Yamane H (2003). Acquisition of 16S r RNA methylase gene in *Pseudomonas aeruginosa*. *Lancet.*, **362**: 1888.

Yoo SS, Cha SI and Shin KM (2010). Bacterial pneumonia following cytotoxic chemotherapy for lung cancer: Clinical features, treatment outcome and prognostic factors. *Scand. J. Infect. Dis.,* **42**(10): 734-740.

Yu F, Wang L and Pan J (2009). Prevalence of 16S rRNA methylase genes in *Klebsiella pneumoniae* isolates from a Chinese teaching hospital: Coexistence of *rmt B* and *armA* genes in the same isolate. *Diagn. Microbiol., Infect. Dis*., **64**(1): 57-63.