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Research Article

PREVALENCE OF COLISTIN RESISTANCE AMONG NON-FERMENTING GRAM NEGATIVE BACTERIA ISOLATED FROM VARIOUS CLINICAL SPECIMENS IN A TERTIARY CARE HOSPITAL

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Abstract- Background and Objectives: Indiscriminate use of antimicrobials has caused emergence of Multidrug Resistance bacteria (MDR). Resistance to several antibiotics including Colistin is great challenge in therapy. NFGNB being common cause of Hospital Acquired Infections and their resistance to various antimicrobial drugs, significantly influences morbidity, mortality and high cost of treatment of infected patients. This study aims to find out the prevalence of colistin resistance among non-fermenting gram-negative bacteria isolated from various clinical specimens received at Bacteriology Laboratory, Civil Hospital, Ahmedabad. Methods: The study was conducted over a period of 1 year from October 2020 to October 2021. Total 41390 samples received. NFGNB were isolated by standard microbiological identification technique. The isolates were subjected to antimicrobial susceptibility testing by Modified Kirby Bauer Disc Diffusion method. Colistin sensitivity is screened on Colistin Screen Agar (CSA) and resistance is confirmed by BMD (Broth Micro Dilution). Result: Among 41390 samples tested, 17491 had positive culture growth, 7610 isolates had gram-negative bacterial growth, out of which 4393 were non fermenters, in which 53 screened for Colistin resistance. Interpretation and Conclusion: This study provides prevalence of colistin resistance among non-fermenting gram-negative bacteria. *P. aeruginosa* causing hospital associated infections shows emergence of resistance to commonly used antimicrobials agents and colistin is last resort to treat MDR organisms. CL resistance in MDR organisms is a serious threat and hence there is a need of awareness among clinicians about judicious use of antibiotics and strict infection control practices needs to be followed.

Keywords- *Colistin, NFGNB, Colistin Screen Agar, Broth Microdilution-BMD*

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Introduction

India is the Indiscriminate use of antimicrobials has caused the worldwide emergence of multidrug resistance to several antimicrobials, including colistin. Asia has been reported with highest rates of this type of resistance [1]. Since colistin is used as last resort to treat infections caused by MDR bacteria, its resistance pose threat in therapy [2]. *Pseudomonas* and *Acinetobacter* are amongst the commonest non fermenters causing infections in a hospital setting. *Pseudomonas aeruginosa* is a skillful microorganism that causes severe opportunistic infections and hospital associated infections (HAI) in immunocompromised patients due to its metabolic versatility [3].

Isolation of *P. aeruginosa* resistant to almost all available antimicrobials is increasing in Intensive Care Units (ICU), leading to an increase in the number of healthcare-associated infections (HAI), significantly influencing morbidity, mortality, and high costs of treatment of infected patients especially those showing multi-drug resistance patterns [1-3]. The emergence of multidrug-resistant (MDR) or extensively drug-resistant (XDR) or pan-drug-resistant (PDR) *P. aeruginosa* becomes a significant public health problem that can lead to delayed antimicrobial therapy or its failure and the increase in the mortality rate especially with the appearance of carbapenem-resistant *P. aeruginosa*. So, attention is required because these resistant strains may show resistance to all available antimicrobials or showed susceptibility only to toxic ones such as colistin or polymyxins leaving no choices for the health-care team in the treatment of severe infections associated with MDR *P. aeruginosa* [4-6].

Colistin, known as polymyxin E, is one member of a family of cationic polypeptide known as polymyxins. The action of polymyxins on bacteria depends mainly on the electrostatic interaction between the positively charged antibiotic and the negatively charged phosphate group of lipid A localized on the outer membrane after its binding, it diffuses through the outer membrane, periplasmic space and interact with the inner membrane, causes destabilization to the outer membrane, pore formation, increase permeability, leakage to cytoplasmic content followed by cell lysis [7]. Colistin resistance mainly occurs due to the chemical modification by the enzymatic addition of phosphor-ethanolamine at the 4'- phosphate group of the lipid A moiety of the lipopolysaccharide decreasing the net-negative charge of the outer membrane resulting in decreasing polymyxin affinity. Resistance to colistin may be resulted from chromosomally encoded mutation as reported in *K. pneumoniae* or the horizontal transfer of resistance by means of plasmid carrying colistin-resistant gene (mcr^{-1}) [7-11]. This study aims to investigate colistin resistance among NFGNB. isolated from different specimens in B.J Medical college, Ahmedabad, also indicating the emergence of untreatable diseases in our area due to the possibility of transferring colistin resistance to highly resistant bacteria.

Materials and Methods

Study Design

This study was done in our hospital which is a tertiary care centre over a period of 1 year from October 2020 to October 2021.All the samples sent for culture to the bacteriology laboratory were included in the study. Samples studied were urine, swabs, sputum, endotracheal aspirate, broncho-alveolar lavage (BAL), pus, blood, CSF (cerebrospinal fluid), pleural fluid, pericardial fluid and ascitic fluid *etc*. Samples from patients of both sexes and all ages were included. All the samples were processed for gram's staining and culture.

Microbiological Analysis

Identification and characterisation of isolates were performed on the basis of gram staining, microscopic characteristics, colony characteristics and biochemical test using standard microbiological methods.

Culture

Culture was done on Blood agar, MacConkey agar and Chocolate agar using standard technique and incubated for 35+/-2°C for 48 hrs, observed every 24 hours of incubation. In case of urine, cultures showing a significant growth 105 CFU / ml were further processed. Routine samples like urine, swabs, sputum, ET aspirates, BAL were cultured on Blood and MacConkey agar.

Precious fluids like, pus, body fluids, tissues, were cultured on blood and MacConkey agar and CSF on chocolate and MacConkey agar. Samples showing no growth were cultured again after addition of BHI and further incubation of at-least 4-6 hours. Blood was received in automated blood culture bottle, which were loaded in BACTEC machine for at-least 5 days and samples showing positive growth were cultured on Chocolate agar and MacConkey agar.

Identification and AST

All the samples were processed for routine bacteriological analysis. Positive growths were subjected to gram staining. Antibiotic susceptibility patterns were done by Kirby Bauer disc diffusion method and automated VITEK®2 system was used for speciation and drug susceptibility. The organism isolated were identified using the appropriate biochemical tests. All the organisms that grew on Triple Sugar Iron (TSI) and produced an alkaline reaction were provisionally considered to be NFGNB and identified further using a standard protocol for identification. The characters assessed included morphology on Gram's stain, motility, pigment production, oxidase production, OF test (Hugh-Leifson medium) for glucose. Antibiotic susceptibility results were expressed as susceptible, intermediate or resistant according to the criteria of Clinical Laboratory Standards Institute MI00S, 31st edition (2021) [9]. All the isolates were screened for Colistin sensitivity is screened on Colistin Screen Agar (CSA). Resistance on CSA is confirmed by BMD (Broth Micro Dilution)

Result

A total of 41390 samples were processed in the study period of 1year (October 2020 to October 2021) and 17491 were culture positive.7610 isolates had gram-negative bacterial growth, out of which 4393 were non fermenters. The most common isolates amongst non-fermenters were *Pseudomonas* sp. (59.38%) *Acinetobacter* sp. (35.37%), followed by Burkholderia group, Sphingomonas, and others [Table-1]. Study population consisted of 2991(68.08%) males and 1402 (31.91%) females. The mean age of patients was 43 years. Swabs from intraoperative and post-operative sites were most common 1151/4393(26.2%) followed by Respiratory tract samples 568/4393(12.9%), urine (479/493), blood samples (413/4393), pleural fluid (193/4393) CSF (191/4393), ascitic fluid (55/4393).

Table-1 *Non-fermenters isolated from patients during the study period (n=4393)*

SN	Isolates	Total
1	*Acinetobacter*	
	Acinetobacter baumannii	1415
	Acinetobacter lwoffii	32
	Acinetobacter spp	103
	Acinetobacter haemolyticus	2
	Acinetobacter juni	2
2	*Pseudomonas*	
	Pseudomonas aeruginosa	2171
	Pseudomonas spp	245
	Pseudomonas flourescens	9
	Pseudomonas putida	5
	Pseudomonas stutzeri	176
3	*Burkholderia cepacian group*	130
4	*Sphingomonas paucimobilis*	97
5	*Elizabethkingia meningoseptica*	3
6	Others	3
		4393

Antibiotic susceptibility pattern

Pseudomonas aeruginosa was the most common species of Pseudomonas isolated. 467 out of 2171 *Pseudomonas aeruginosa* isolates were MDR.

Discussion

The isolation rate of non-fermenters was 4393/41390(10.6%) in this study. This is almost similar to a 12.8%isolation rate reported by Rit *et al*. [9]. Nearly one fourth of the gram-negative bacterial infections in hospital setting were caused by non-fermenters according to this study. This is much higher as compared to recently reported results (11.6%) by Grewal *et al*. *Pseudomonas* and *Acinetobacter* were the most common non fermenters isolated. We observed that *Pseudomonas* was the most common isolate (59.38%) amongst non-fermenters, *Pseudomonas* as found by studies published in 2009 and 2013 [5,9]. Colistin resistance was found to be 0.14% in *Acinetobacter baumanii* in the present study. World over the colistin resistance in *Acinetobacter* varies from region to region and amongst different patient groups. Colistin resistance rates as less as 1.4% from Brazil to as high as 40% from Spain have been reported [12,13].

Table-2 *Antibiotic resistance amongst Pseudomonas and Acinetobacter*

SN	Isolates	Number of Isolates	MDR	Resistant to Colistin
1	*Acinetobacter*			
I(a)	*Acinetobacter baumannii*	1415	329	2
I(b)	*Acinetobacter lwoffii*	32	2	0
I(d)	*Acinetobacter haemolyticus*	2	0	0
2	*Pseudomonas*			
2(a)	*Pseudomonas aeruginosa*	2171	467	2
2(b)	*Pseudomonas putida*	5	0	0
2(c)	*Pseudomonas stutzeri*	176	12	1
2(f)	*Pseudomonas* spp	245	19	2
	Total	4046	975	7
			-24.09%	-0.17%

23.25% of *Pseudomonas aeruginosa* isolates were found to be MDR. Various studies have explored susceptibility of *Pseudomonas aeruginosa* to imipenem and the resistance rates ranging from 8.2% to 90% have been reported in the past five years from different regions of India [9,14,15]. Agarwal, *et al*. [11] recently published imipenem resistance rates of 52% in *Pseudomonas* and 90% in *Acinetobacter*, which resonates with our results. Colistin resistance rates in *Pseudomonas* were same as in *Acinetobacter* in the present study. High resistance to carbapenems is leading to increased use of colistin as a therapeutic agent in many hospitals including ours which in turn is leading to development of colistin resistance. There are multiple mechanisms of colistin resistance in *Pseudomonas* and *Acinetobacter* and the understanding of the same is still evolving. The most important mechanisms of colistin resistance in *Acinetobacter baumannii* are loss of LPS modification of lipid A with phosphor ethanolamine and glycosylation of Lipid A with hexosamine [16-18]. Mechanisms of colistin resistance in *Pseudomonas aeruginosa* are alteration of LPS composition, overexpression of outer membrane protein OprH and activation of LPS modifying operons by mutations in two component systems [18,19]. Qureshi *et al*. [20] concluded that colistin-resistant *Acinetobacter baumannii* occurred almost exclusively among patients who had received colistin methansulfonate for treatment of carbapenem-resistant, colistin-susceptible *Acinetobacter baumannii* infection and Lipid A modification by the addition of phosphor ethanolamine accounted for their colistin resistance. Therefore, colistin usage appears to be the single most important driving force for the development of colistin resistance.

MDR non-fermenters are a serious threat in almost every hospital setting now, compounded by the ever- increasing colistin resistance. Non-fermenters can spread from one patient to another and are capable of causing outbreaks of serious infections. Resource limited countries like India struggle to keep up with ideal hospital infection control protocol, so it is even more dangerous in such settings. The cornerstones of such an approach are judicious use of colistin and strict antibiotic stewardship.

Recent CLSI guidelines recommend that for the treatment of *Pseudomonas* and *Acinetobacter baumannii* complex, Colistin should be administered with a loading dose and at the maximum recommended doses, in combination with other agents [21]. Antimicrobial stewardship which is a coordinated program that promotes the appropriate use of antimicrobials, improves patient outcomes, reduces microbial resistance and decreases the spread of infections caused by multidrug organisms. Our main objective should be to improve awareness and understanding of antimicrobial resistance. Reduction of incidence of infection through effective sanitation, hygiene and infection prevention measures. Optimization of use of antimicrobial medicines. And most importantly strengthen the knowledge and evidence base through surveillance and research. Hence studies like these plays a pivotal role in achieving these objectives of antimicrobial stewardship program. Therefore, continuous efforts towards curtailing colistin resistance must be in place lest we are thrown back to the pre-antibiotic era.

In case of *Pseudomonas aeruginosa*, 467/2171(21.5%) isolates were MDR. It was observed that 2169 isolates were susceptible,2/2171 were resistant (MIC16µg/ml) to colistin. All Isolates of Burkholderia, Elizabethkingia and Sphingomonas were resistant to colistin.

Conclusion

Increasing role of non-fermenters as pathogens in the hospital settings is worrying. Judicious use of antibiotics is needed to curb the high antibiotic resistance amongst non-fermenters.

Application of research: This study provides prevalence of colistin resistance among non-fermenting gram-negative bacteria. P. aeruginosa causing hospital associated infections shows emergence of resistance to commonly used antimicrobials agents and colistin is last resort to treat MDR organisms. CL resistance in MDR organisms is a serious threat and hence there is a need of awareness among clinicians about judicious use of antibiotics and strict infection control practices needs to be followed

Research Category: Medical Microbiology

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Study area / Sample Collection: Tertiary Care Hospital, Ahmedabad, Gujarat

Strain name: *P. aeruginosa*

Conflict of Interest: None declared

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