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COLISTIN RESISTANCE GRAM NEGATIVE BACTERIA FROM TRACHEAL ASPIRATES AMONG PATIENTS ADMITTED IN INTENSIVE CARE UNIT OF A TERTIARY CARE HOSPITAL, BANGLADESH

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ABSTRACT

Background: Multidrug-resistant (MDR) Gram negative bacteria (GNB) poses a serious therapeutic problem as no development of newer antimicrobials. In most cases, colistin remains the last viable effective option which leads to an increase exposure of patients to colistin resulting the growing threat of colistin resistance. **Objective:** The aim of this study was to observe antibiotic susceptibility pattern and prevalence of colistin resistance among gram negative bacteria from tracheal aspirates among patients admitted in ICU of a tertiary care hospital. **Materials and Methods:** A total of 160 specimens of tracheal aspirates collected from ICU patients were cultured on appropriate media in the department of microbiology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Bangladesh. Then standard biochemical tests were done to identify the isolated organism. Antibiotic susceptibility profile of isolated bacteria was studied to detect their resistant pattern against colistin. **Results:** *Acinetobacter spp.* was the predominant organism (40.59%) found in tracheal aspirate. Among the isolated gram negative bacteris 10.7% were found resistant to colistin. **Conclusion:** The distribution of colistin resistance pattern in the study is quite unnerving. Producing a local antibiogram database will improve the knowledge of antimicrobial resistance pattern in ICU and will also help to improve treatment strategies.

KEYWORDS: Colistin, intensive care unit, tracheal aspirates, gram negative bacteria, antibiotic susceptibility pattern, *Acinetobacter spp.*

1. INTRODUCTION

An intensive care unit (ICU) provides the critical care and life support for acutely ill and injured patients in a specialized hospital. Hospital acquired infection (HAI) are the major cause of mortality and morbidity among the intubated patients in ICU. It is reported that mortality and morbidity rate by HAI is more among intubated patients in ICU (50%) than among patients in general wards (5%-10%).[1] Equipment's which are frequently used in ICU such as endotracheal tube, intra venous catheter etc. are mostly responsible for HAI.[2] Tracheal intubation also causes colonization in trachea by different bacteria that may responsible for increases the risk of mortality due to superinfections.[3]

This situation is increasing the concern regarding the rise of bacterial resistance to various antimicrobial agents and is becoming a major public health issue. Now a days, the major concern is inappropriate treatment against MDR GNB due to unavailability of newer antibiotics.[4,5]

As a result, there is reemergence of older antibiotics like colistin.[6,7,8] Though colistin was first discovered in the 1940s, it was in use against problematic gram-negative bacteria until the late 1950s.[4,5] Clinical use of colistin by physician was abandoned due to its significant renal and neurological toxicity and replaced by other less toxic antibiotics such as aminoglycosides.[7,8] If large doses of aminoglycosides are given to patients with underlying renal disease over prolonged periods, nephrotoxicity is more likely to occur.[9] Also some studies have been reported that polymyxin particularly colistin remains the last resort among patient with cystic fibrosis, cancer and in burn unit where the use of aminoglycosides become ineffective due to development of resistance.[10,11,12] At the same time, with emergence of MDR and pan drug-resistant (PDR) gram negative bacilli, there is revival of colistin.[13,14] In some cases colistin is the only antimicrobial against pathogens such as carbapenem-resistant *Acinetobacter spp* and carbapenemase producing *Pseudomonas aeruginosa* and *Klebsiella*

pneumoniae which causes selective pressure of its extensive use and may lead to the emergence of resistance. In addition, superinfection with pathogens including Proteus, Providencia, Serratia and Morganella species those are intrinsically resistant to colistin may also be a matter of concern.[15] Thus, with widespread use of colistin, there are reports of rapidly rising resistance against this antibiotic.[16] Falagas ME et al (2007) demonstrated the prevalence of colistin resistance ranging from 1.9% - 3.3%.[17] A very high colistin rate of 13.1% in *A. baumannii* was reported by a Spanish study (2001).[18] Another study of Kuwait reported12% colistin resistance among Acinetobacter isolates.[19] Gales et al (2006) reported 1.3%, 2.1% and 1.8% polymixin resistance against *Pseudomonas spp*, *Acinetobacter spp* and *Klebsiella spp* respectively.[20] This study was designed to find out the resistance pattern of colistin from tracheal aspirates among ICU patients in a tertiary care hospital, Bangladesh.

2. MATERIALS AND METHODS

2.1. Study Design and setting

This Laboratory based cross-sectional study was carried out at Department of microbiology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh from January 2016 to June 2016. One hundred and sixty samples of tracheal aspirates were collected from each of 160 ICU admitted patient with tracheal intubation irrespective of age and sex .The samples were then sent to the department of microbiology for laboratory procedure. Repeat collection of sample from the same patient was excluded to avoid duplication of isolates.

2.2. Specimen collection

At least 1 ml of tracheal aspirate was aseptically collected in a sterile falcon tube with the help of suction catheter by attending physician using standard aspiration technique.[21] Then the aspirated material was transported to the laboratory for microbiological study.

2.3. Sample processing and Identification of organisms

After sample collection all the steps including sample processing, smear preparation and culture were done in a Biosafety level 2. The aspirates were liquefied and homogenized in a sterile tube then centrifugation was done at 2000 x g for 10 minutes.[22] After that deposits were inoculated in bacterial culture media such as blood agar medium, chocolate agar medium and MacConkey agar medium (three sectors consecutively) and incubated overnight aerobically at 37°C. Depending on the number of colonies on bacterial culture media microbial growth was categorized as rare, light, moderate and heavy growth (Table I).[23,24] Moderate to heavy growth was regard as significant.[25] Characterization and confirmation of isolated organism are done by Gram staining and standard biochemical tests. To identify bacteria of interest, several biochemical tests such as, Triple Sugar Iron (TSI) Test, Citrate Utilization Test, Motility Indole Urease (MIU) Test, Oxidase Test, Catalase Test, Coagulase Test were performed according to the manual of methods for general bacteriology by American society of Microbiology.[26]

2.4. Antimicrobial sensitivity testing

For antimicrobial sensitivity testing Mueller-Hinton agar medium was used. Kirby-Bauer modified disc-diffusion methods were employed to determine the antibiotic resistant pattern for the isolated organisms.[23] Following thirteen Antibiotic discs from Oxoid Ltd. UK were used: cotrimoxazol (25 µg⁄disc), ciprofloxacin (5 µg⁄disc), ceftriaxone (30 µg⁄disc), gentamicin (10 µg⁄disc), cefotaxime (10 µg⁄disc), ceftazidime (30 µg⁄disc), amikacin (30 µg⁄disc), aztreonam (30 µg⁄disc) imipenem (10 µg⁄disc), netilmicin (30 µg⁄disc), piperacillin-tazobactam (110 µg⁄disc), ticarcillin (75 µg⁄disc), colistin (30 µg⁄disc). The zone diameters of each drug were interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI 2018)

2.5. Data analysis

After collection all data were checked and edited. Then all statistical analysis was performed using 'Microsoft Office Excel 2007' program.

3. RESULTS

3.1. Isolated Pathogens

One hundrad sixty of tracheal aspirates from 160 ICU patients were enrolled in this study. Among them 92 (57.5%) were male and 68 (42.5%) were female. The mean age of the study subject was 44.17± 1.42 years. Out of 160 specimens submitted, 101(63.12%) specimens were found to be positive in cultures. Among the 101 culture positive samples, 93(92.07%) were Gram negative bacilli (GNB) and 7.93% were Gram positive bacteria. Among the GNB most common pathogen was *Acinetobacter spp.* (40.59%) followed by *Klebsiella spp.* (23.76%), *Pseudomonas spp.* (16.83%) and *E coli* (10.89%) respectively (Table II).

3.2. Antimicrobial Susceptibility

Here for GNB 13 types of antibiotics disks were uses for sensitivity. Antimicrobial susceptibility revealed that *Acinetobacter spp.* mostly showed resistant to ceftriaxone, ceftazidime and amikacin with 97.56% followed by gentamicin (95.12%) and ciprofloxacin (92.68%) respectively. *Klebsiela spp.* showed resistant to ciprofloxacin, piperacillin-tazobactam and ticarcillin with 91.6%. *Psedomonas spp.* was resistance to aztreonam with 88.23%, gentamicin and amikacin with 82.35%. The data for the microbial resistance pattern is shown in (Table III).

Four out of the 24strains of *Klebsiella spp*. (16.6%) was found to be resistant to colistin . *Pseudomonas* spp. and *Acinetobacter spp* was found to be resistant to colistin with 11.76% and 9.75 respectively (Table III).

Total 93 Gram negative bacilli were isolated in this study period. While 83 (89.24%) were sensitive to colistin and 10 (10.76%) were resistant. (Table IV).

Table I: Semi-quantitative reporting of microbial growth in culture performed in petri-dishes (Fujitani S *et al*. 2010).

Report	Number of colonies in each sector		
	1st	2nd	3rd
Rare	<10	0	0
Light	≥10	<5	0
Moderate	≥10	≥5	<5
Heavy	≥10	≥5	≥5

- The semi-quantitative scoring was determined by the four-quadrant method and classified as follows: 0 = no growth; 1+ = rare growth; 2+ = light growth; 3+ = moderate growth; 4+ = heavy growth.
- Cultures showing a moderate to heavy growth with 3+ or 4+ grades were considered as positive.
- The growth of the organisms on culture plates was recorded as rare (≤10 colonies in the first quadrant) or as 1+to 4+ (>10 colonies in the first quadrant to >10 colonies in all quadrant)

Table II: Percentage of bacterial isolates in culture positive tracheal aspirates of ICU patients.

Bacterial isolate (n=101)		Number (n)	Percentage (%)
Gram negative bacteria (n=93)	*Acinetobacterspp*	41	40.59
	Klebsiellaspp	24	23.76
	Pseudomonas spp	17	18.83
	E coli	11	10.89
Gram positive bacteria (n=08)	*Staph aureus*	06	5.94
	S pneumonia	02	1.98

Table III: Antibiotic resistance pattern of GNB to different antibiotics.

Drug name	*Acinetobacter spp* N=41	*Pseudomonas spp* N=17	*Klebsiella spp* N=24	*E coli* N=11
Cotrimoxazole	38 (92.6)	NA	21 (87.5)	9 (81.8)
Ciprofloxacin	38 (92.6)	12 (70.5)	22 (91.6)	8 (72.7)
Ceftriaxone	40 (97.5)	NA	24 (95.8)	2 (18.1)
Gentamicin	39 (95.1)	14 (82.3)	20 (83.3)	2 (18.1)
Cefotaxime	39 (95.1)	NA	23 (95.8)	3 (27.2)
Ceftazidime	40 (97.5)	12 (70.5)	23 (95.8)	4 (36.3)
Amikacin	40 (97.5)	14 (82.3)	20 (83.3)	2 (18.1)
Aztreonam	39 (95.1)	15 (88.2)	23 (95.8)	4 (36.3)
Imipenem	38 (92.6)	10 (58.8)	19 (79.1)	1 (9.0)
Netilmicin	38 (92.6)	12 (70.5)	20 (83.3)	1 (9.0)
Piperacillin-tazobactam	36 (87.8)	12 (70.5)	22 (91.6)	2 (18.1)
Ticarcillin	38 (92.6)	12 (70.5)	22 (91.6)	2 (18.1)
Colistin	4 (9.75)	2 (11.7)	4 (16.6)	00

NA=Not Applicable

Table IV: Colistin resistance among isolated Gram negative bacteria from tracheal aspirates of ICU patients.

Bacterial isolates	Colistin	
	Sensitive n(%)	Resistant n(%)
Acinetobacter spp (n =41)	37(90.25)	04(9.75)
Klebsiella *spp* (n =24)	20(83.4)	04(16.6)
Pseudomonas spp (n =17)	15(88.3)	02(11.7)
E coli (n=11)	11(100)	0
Total (%)	83(89.24)	10(10.76)

4. DISCUSSION

Emergence of resistance to β-lactam, aminoglycoside or quinolone by multidrug resistance bacteria has forced to think about polymyxins, especially colistin.[8,27] Due to its nephrotoxic activity colistin was replaced in the 1970s with other relatively less-toxic aminoglycosides and other antipseudomonal agents.[8,28] Due to reemergence of MDR bacteria, colistin has been started to reuse recently all over the world.[6,29] After the frequent use of colistin, there have been reports of colistin resistance strains within years.[30]

There are plenty of laboratory studies on the prevalence of colistin resistance. A review report among eleven laboratory studies showed the prevalence of colistin resistance being ranging from 1.9% - 3.3%[17] among GNB. Chand Wattal et al showed that 8% of the

Pseudomonas aeruginosa were colistin resistant in North India.[31] In another study from Kuwait, 12% of colistin resistance among *Acinetobacter baumannii* was observed.[32] A series of 13 patients has been reported with colistin resistance from South India.[16] In this study, *Klebsiella spp.* was found highest resistance to colistin with 16.6%. *Pseudomonas spp*. and *Acinetobacter spp* have resistance against colistin with 11.76% and 9.75% respectively. Presence of this resistance in critically ill patients in ICU was alarming. Finding shown in other study that some species such as *Acinetobacter baumannii* strain only susceptible to polymyxins, which may cause longterm use of colistin have become a common problem especially in intensive care unit.[33] These finding shows that restriction of colistin use should be implemented to control the rate of colistin resistance bacteria.

CONCLUSION

Extensive use of colistin in the treatment of MDR-GNB resulting development of resistance towards colistin. Almost 10.76% of the clinical isolates were resistance to colistin in present study which is a worrisome therapeutic scenario. Prolong ICU stay and history of prior exposure to colistin likely the aggravating factor for the development of colistin resistance GNB. Microbiologist, consultant and hospital infection control committee should work together to prevent further rise in the drug resistance against this last resort of antimicrobials. A multidisciplinary approach must be implemented with restricted and rational use of the colistin to control this situation. This is a matter of great concern as the last resort to treat multi-drug resistant Gram-negative bacilli will lose its effectiveness.

Ethics Approval and Consent to Participate

Ethical approval was not required to carry out this work as the bacterial isolates were collected as part of routine patient care investigation in the hospital.

Human and Animal Rights

No Animals/Humans were used for studies that are base of this research.

Consent for Publication: Not applicable.

Conflict of Interest: This article contents no conflict of interest.

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