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Colistin Resistance amongst Non-Fermenters in the Hospital Setting: A Lurking Threat

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Abstract

Introduction: Non-fermenters have gained significance as etiological agents of mild to potentially life threatening healthcare associated infections in recent years. Pseudomonas and Acinetobacter are amongst the commonest non fermenters causing infections in a hospital setting. The aim of this study was to find the prevalence of colistin resistance in Pseudomonas and Acinetobacter isolates from inpatients. *Methodology:* This retrospective study was done in our hospital which is a tertiary care centre, from April 2016 to March 2017. Identification and antibiotic susceptibility testing was done by VITEK®2 system (BioMerieux, North Carolina/USA). Antibiotic susceptibility results were interpreted according to the criteria of Clinical Laboratory Standards Institute M100S (26th edition). Patient information and microbiological profile of the organism isolated was recorded. For statistical analysis, data was described in terms of fractions and percentages and percentages were used to compare the data in two sets. *Results:* The most common isolates amongst non fermenters were Acinetobacter sp.(45.93%), and Pseudomonas sp. (41.62%). 92.13% of the Acinetobacter baumannii isolates were MDR and 88.76% were resistant to carbapenems. Sixty-four percent of Acinetobacter baumannii isolates were susceptible to colistin and tigecycline only and 7.86% were resistant to colistin. In case of Pseudomonas aeruginosa, 74.67% isolates were MDR and 56% were resistant to carbapenems. Nearly 77.33% isolates were susceptible, 5.33% were intermediate 17.34% were resistant to colistin. The colistin resistant isolates of Acinetobacter baumannii and Pseudomonas aeruginosa were predominantly isolated from endotracheal aspirate (57.14%) and pus (61.53%) samples respectively. Overall 5 out of 23 (21.74%) colistin resistant isolates were resistant only to colistin and were susceptible to all other antibiotics tested. *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.

Keywords: Colistin Resistance; Acinetobacter; Pseudomonas; Non Fermenters.

Introduction

Non fermenters are taxonomically diverse gram negative bacilli which utilise glucose oxidatively or not at all. They exist as environmental saprophytes or commensals in the human gut [1,2]. Lately, they have gained much significance as etiological agents of mild to potentially life threatening healthcare associated infections. These infections include device related infections, urinary tract infections, surgical site infections, pneumonia, bacteremia and sepsis [3,4]. *Pseudomonas and Acinetobacter* are amongst the commonest non fermenters causing infections in a hospital setting [5].

Antibiotic resistance amongst non-fermenters is an increasingly menacing situation. *Pseudomonas*

and *Acinetobacter* are increasingly becoming resistant to cephalosporins, carbapenems and other drugs commonly used for treating infections caused by them [6]. Colistin is a commonly used therapeutic drug for MDR *Pseudomonas* and *Acinetobacter* and is a drug of last resort in many such cases [7]. Due to this increased use, colistin resistance in these organisms has surfaced. High rates of colistin resistance in *Pseudomonas* and *Acinetobacter* have been reported in recent times [8].

Therefore, it is important that the antibiotic susceptibility profile of non-fermenters in a hospital is known and the antibiotic policy is updated accordingly. The aim of this study was to find the prevalence of colistin resistance in *Pseudomonas* and *Acinetobacter* isolates from inpatients.

Materials and methods

Study design

This retrospective study was done in our hospital which is a tertiary care centre in western India over a period of one year (April 2016 to March 2017). All the samples from inpatients sent for culture to the microbiology laboratory were included in the study. Samples studied were sputum, endotracheal aspirate, broncho-alveolar lavage (BAL), pus, urine, blood, CSF (cerebrospinal fluid), pleural fluid, pericardial fluid and ascitic fluid. Samples from patients of both sexes and all ages were included. Samples from patients with HIV and patients receiving immunosuppressive drugs were excluded from the study. Repeated isolates from a single patient were excluded from the final analysis. patient records were reviewed for relevant details.

Microbiological analysis

Culture: Culture was done on Blood agar and MacConkey agar (Hi-Media, Mumbai, India) using standard technique. In case of urine, cultures showing a significant growth of $\geq 10^5$ CFU/ml were further processed. Cultures with mixed growth on the culture media were excluded.

Identification and AST: Identification of isolates and analysis of their antibiotic susceptibility patterns was done by automated VITEK®2 system (BioMerieux, North Carolina/USA), as per the manufacturer's instructions. Antibiotic susceptibility results were expressed as susceptible, intermediate or resistant according to the criteria of Clinical Laboratory Standards Institute M100S, 26th edition (2016) [9]. For the purpose of quality control, *Escherichia coli* (ATCC 25922) was used.

MDR: Isolates resistant to three or more classes of antibiotics were considered as multi drug resistant (MDR).

CRB: Isolates resistant to both Imipenem and Meropenem were considered as Carbepenem Resistant Bacteria(CRB).

Statistical analysis

Data was described in terms of fractions and percentages. Percentages were used to compare the data in two sets.

Ethical considerations: The study was approved by the institutional ethics committee (SVIEC/ OW/17014)

Results

Baseline characteristics

A total of 1630 samples from inpatients were processed in the study period and 947 were culture positive. Seven hundred and eighty-four isolates had gram-negative bacterial growth, out of which 209 were non fermenters. The most common isolates amongst non fermenters were *Acinetobacter* sp. (45.93%), *Pseudomonas* sp. (41.62%) followed by *Burkholderia cepacia* group, *Sphingomonas*, *Myroides* and others [Table1].

Study population consisted of 134 (64.11%) males and 75 (35.89%) females. The mean age of patients was 42 years. Samples with non-fermenter isolation were received from ICU, Surgical wards, Orthopaedics wards, OT recovery ward, NICU, PICU, Neurosurgical ICU and respiratory medicine wards amongst others [Figure 1]. Respiratory tract samples were the most common (98/209) followed by pus samples (79/209), blood samples (15/209), urine (8/209), ascitic fluid (5/209), pleural fluid (2/209) and CSF (2/209). Out of the 98 respiratory tract samples, 55 were endotracheal aspirates, 40 were sputum samples and 2 were BAL samples. Fifty three patients were diagnosed with Ventilator associated pneumonia (VAP), 68 with surgical site infections (SSI), 14 with Blood stream Infection (BSI), 8 with UTI, and 2 with meningitis.

Antibiotic susceptibility pattern

Acinetobacter baumannii was the most common species of *Acinetobacter* isolated. Eighty two out of 89 (92.14%) *Acinetobacter baumannii* isolates were MDR and 79/89 (88.76%) were resistant to carbapenems [Table 2]. A total of 57/89 (64%) were susceptible to colistin and tigecycline only.

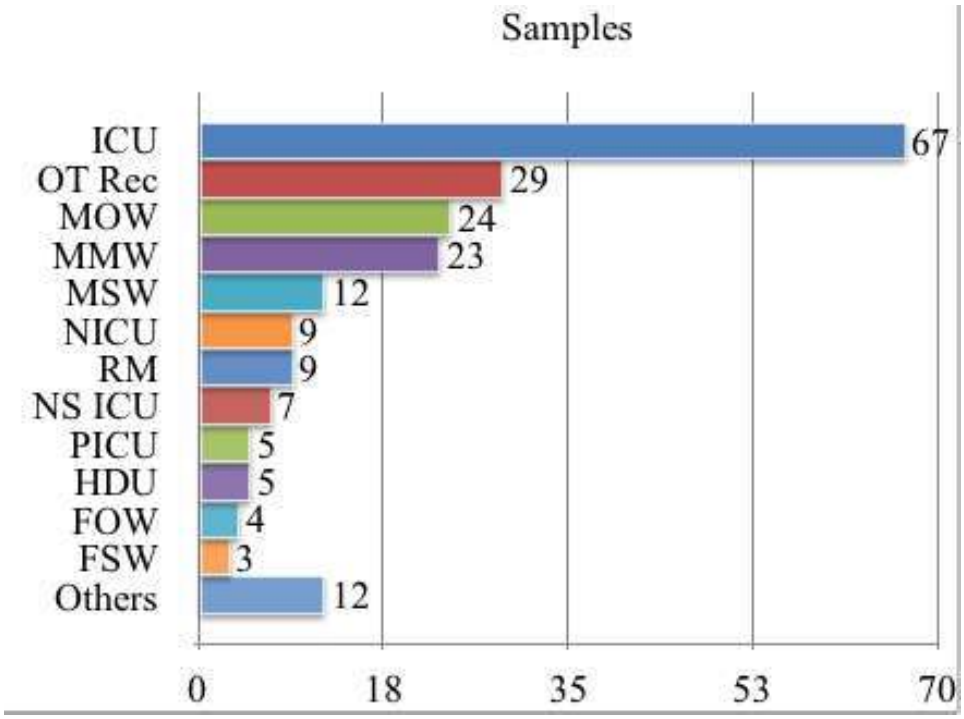

Fig. 1: Distribution of NFGNB isolation from wards in which patients were admitted during the study period.

ICU= Intensive care unit, OT Rec = OT recovery, MOW= Male orthopaedics ward, MMW= Male medicine ward, MSW= Male surgical ward, NICU= Neonatal intensive care unit, RM= Respiratory medicine, NS ICU= Neurosurgery intensive care unit, PICU= Paediatric intensive care unit, HDU= high dependency unit, FOW= Female orthopaedics ward, FSW= Female surgical ward.

Table 1: Non fermenters isolated from inpatients during the study period (n=209).

S.No	Isolate	Total
1	Acinetobacter	
	Acinetobacter baumannii	89
	Acinetobacter lwoffii	4
	Acinetobacter junii	2
	Acinetobacter haemolyticus	1
2	Pseudomonas	
	Pseudomonas aeruginosa	75
	Pseudomonas flourescens	6
	Pseudomonas putida	2
	Pseudomonas mendocina	2
	Pseudomonas stutzeri	1
	Pseudomonas luteola	1
3	Burkholderia cepacia group	12
4	Sphingomonas paucimobilis	6
5	Myroides sp.	3
6	Elizabethkingia meningoseptica	2
7	Others	3
		209

Table 2: Antibiotic resistance amongst Pseudomonas and Acinetobacter(n=183)

S.No	Isolate	Number of Isolates	MDR	CRB	Resistant to Colistin	Susceptible to Colistin
1	Acinetobacter					
1(a)	Acinetobacter baumannii	89	82 (92.14%)	79 (88.76%)	7 (7.86%)	82 (92.14%)
1(b)	Acinetobacter lwoffii	4	2 (50%)	1 (25%)	0 (0)	4 (100%)
1(c)	Acinetobacter junii	2	0 (0)	0 (0)	0 (0)	2 (100%)
1(d)	Acinetobacter haemolyticus	1	1 (100%)	1 (100%)	0 (0)	1 (100%)
2	Pseudomonas					
2(a)	Pseudomonas aeruginosa	75	56 (74.67%)	42 (56%)	13 (17.34%)	58 (77.33%)
2(b)	Pseudomonas flourescens	6	3 (50%)	3 (50%)	2 (33.34%)	4 (66.66%)
2(c)	Pseudomonas putida	2	1 (50%)	1 (50%)	0 (0)	2 (100%)
2(d)	Pseudomonas mendocina	2	0 (0)	0 (0)	0 (0)	2 (100%)
2(e)	Pseudomonas stutzeri	1	1 (100%)	0 (0)	0 (0)	1 (100%)
2(f)	Pseudomonas luteola	1	0 (0)	0 (0)	1 (100%)	0 (0%)
	Total	183	146 (79.78%)	127 (69.40%)	23 (12.57%)	156 (85.24%)

MDR= Multi Drug Resistant, CRB= Carbapenem Resistant Bacteria

It was observed that 82/89 (92.14%) isolates were susceptible to colistin and 7/89 (7.86%) isolates were resistant to colistin with an MIC of 4µg/ml in 2 cases and ≥16µg/ml in 5 cases. However, amongst the 82 susceptible isolates, 5 (6.1%) had a higher MIC of 2µg/ml as compared to the rest with MIC ≤0.5µg/ml.

In case of *Pseudomonas aeruginosa*, 56/75 (74.67%) isolates were MDR and 42/75 (56%) were resistant to carbapenems [Table 2]. Eighteen of 75 (24%) isolates were susceptible to colistin only. It was observed that 58/75 (77.33%) isolates were susceptible, 4/75 (5.33%) were intermediate (MIC=4µg/ml) and 13/75 (17.34%) were resistant (MIC ≥16µg/ml) to colistin. Out of the 58 sensitive isolates, 56 had MIC≤0.5µg/ml and 4 had an MIC of 2µg/ml. One third (2/6) of *Pseudomonas flourescens* and 1/1 (100%) of *Pseudomonas luteola* isolates were resistant to colistin (MIC≥16µg/ml). All Isolates of *Burkholderia, Myroides, Elizabethkingia* and *Sphingomonas* were resistant to colistin

Characteristics of the colistin resistant isolates

Colistin resistant isolates of *Pseudomonas aeruginosa* were predominantly isolated from patients with SSI (8/13-61.53%), VAP(3/13-23.07%), BSI (1/13-7.7%) and UTI (1/13-7.7%). It was observed that 11/13 (84.61%) of the colistin resistant isolates of *Pseudomonas aeruginosa* were multi drug resistant and 10/13 (76.92%) were resistant to carbapenems. Highest concomitant resistance was seen to tigecycline, ciprofloxacin and TMP-SMX (92.30% each). However, 5/13 (38.46%) isolates were susceptible to cefepime [Table 3].

One colistin resistant isolate of *Pseudomonas fluorescens* was concomitantly resistant to all other antibiotics tested and another was susceptible to tigecycline and TMP-SMX only. The colistin resistant isolate of *Pseudomonas luteola* was susceptible to all other antibiotics tested.

The colistin resistant isolates of *Acinetobacter baumannii* were isolated from patients with VAP (4/7-57.14%), SSI (2/7-28.57%) and BSI (1/7-14.29%). Five out of 7 (71.42%) were multi drug resistant and 4/7 (57.14%) were resistant to carbapenems. High concomitant resistance to amoxycillin-clavulinic acid, amikacin, ciprofloxacin, aztreonam and TMP-SMX (71.42% each) was observed. However unlike *Pseudomonas aeruginosa*, 5/7 (71.42%) isolates were susceptible to tigecycline [Table 3].

Overall 5 out of 23 (21.74%) colistin resistant isolates were resistant only to colistin and were susceptible to all other antibiotics tested. These

Table 3: Antibiotic susceptibility patterns of colistin resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*

S.no	Antibiotic tested	Acinetobacter baumannii(n=7)			Pseudomonas aeruginosa(n=13)		
		S	I	R	S	I	R
1	Amoxicillin-clavulinic acid	2	0	5	2	0	11
2	Piperacillin -tazobactam	3	0	4	2	2	9
3	Cefepime	4	0	3	5	2	6
4	Imipenem	3	0	4	3	0	10
5	Meropenem	2	1	4	3	0	10
6	Amikacin	2	0	5	2	0	11
7	Gentamicin	1	2	4	2	0	11
8	Ciprofloxacin	2	0	5	1	0	12
9	Tigecycline	5	0	2	1	0	12
10	Aztreonam	2	0	5	3	0	10
11	TMP-SMX	2	0	5	1	0	12

S= Sensitive, I= Intermediate, R=Resistant.

included 2 *Acinetobacter baumannii* (MIC>16ug/ml), 2 *Pseudomonas aeruginosa* (MIC>16ug/mg) and 1 *Pseudomonas luteola* (MIC=8ug/ml) isolates. These were isolated from pus (3), blood (1) and endotracheal aspirate (1).

Discussion

The isolation rate of non fermenters was 12.82% (209/1630) in this study. This is similar to a 12.8% isolation rate reported by Rit et al. [10]. 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. [11]. This is probably because their study included analysis of all the isolates including outpatients and inpatients whereas our study focussed only on inpatients. *Acinetobacter* and *Pseudomonas* were the most common non fermenters isolated. We observed that *Acinetobacter* was the most common isolate (45.93%) amongst non fermenters as opposed to *Pseudomonas* as found by studies published in 2009 and 2013 [5,10]. However in a study published from Dehradun, India, in 2017, *Acinetobacter* was the most common non fermenter isolated (63.63%) followed by *Pseudomonas*, similar to our study [12].

Our results show that 92.13% of the *Acinetobacter baumannii* isolates were MDR and 88.76% were CRB. This rate of MDR *Acinetobacter baumanii* is much higher than that reported by Grewal et al. [11] (64.71%) from India and Cai et al. [13] (72.23%) from China. This could be attributed to different patient populations studied. Other studies from India [12,14] have however reported a high resistance to carbapenems (90.5%-100%) in *Acinetobacter baumanii*, similar to our results.

Colistin resistance was found to be 7.86% 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 [15,16]. Taneja et al. [17] reported 3.5% colistin resistance in *Acinetobacter baumanii* in 2011. However, in a more recently published study (Behera et al. 2017) [18], the resistance rate was 7% in *Acinetobacter baumanii*, similar to our study results. Interestingly, Gupta et al. [14] reported 53% resistance to colistin in *Acinetobacter baumanii* isolated from ventilator associated pneumonia patients.

Three-fourth (74.67%) of *Pseudomonas aeruginosa* isolates were found to be MDR and more than half (56%) were CRB in our study. 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 [10,19,20]. Agarwal S. et al. [12] recently published imipenem resistance rates of 52% in *Pseudomonas* and 90% in *Acinetobacter*, which resonates with our results.

Colistin resistance rates in Pseudomonas were much higher (17.34%) than *Acinetobacter* (7.86%) in the present study. Colistin resistance in *Pseudomonas aeruginosa* is also variable in reports from different Indian hospitals. Wattal et al. [6] reported 8% colistin resistance in ICU samples from north India whereas Ramesh et al. [21] reported colistin resistance of 30% from two south Indian hospitals. 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 phosphoethanolamine and glysosylation of Lipid A with hexosamine [22,23,25]. 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 [24,25]. Qureshi et al. [26] 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 phosphoethanolamine accounted for their colistin resistance. Therefore colistin usage appears to be single most important driving force for the development of colistin resistance. Nearly 20% of our colistin resistant isolates were resistant only to colistin and susceptible to all other antibiotics tested. This may have been driven by excess use of colistin by clinicians in the hospital setting and is a worrisome trend.

MDR non fermenters are a lurking 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 protocols, so it is even more dangerous in such settings. Thus the need to reduce colistin resistance cannot be emphasized more. The cornerstones of such an approach are judicious use of colistin and strict antibiotic stewardship. De-escalation after obtaining culture results is of paramount importance. 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 [27]. Therefore, continuous efforts towards curtailing colistin resistance must be in place lest we are thrown back to the pre-antibiotic era.

Limitations of the study: Molecular tests to elucidate mechanisms of resistance in the colistin resistant isolates especially those only resistant to colistin, were unavailable at our institute. Therefore the mechanism of colistin resistance could not be commented upon.

Future directions: Larger longitudinal studies to monitor the epidemiology and mechanisms of colistin resistance amongst non fermenters may be taken up. It will be very useful if the trend of colistin resistance is monitored with respect to actual colistin usage in the hospital setting.

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.

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