COLISTIN RESISTANCE AMONG GRAM-NEGATIVE NON FERMENTORS ISOLATED FROM PATIENTS AT A TERTIARY CARE REFERRAL BURN CENTRE

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ABSTRACT

Objectives: Colistin has been increasingly used for the treating infections caused by carbapenem resistant bacteria. Resistance to colistin is increasingly being reported among carbapenem–resistant Enterobacteriaceae as well as *Acinetobacter* and *Pseudomonas* species. This study was undertaken to determine the frequency of colistin resistance among fermenter and non-fermenter Gram-negative rods in our setup.

Methods: The present study was conducted in the Microbiology section of Pathology Laboratory of Jinnah Burn and Reconstructive Surgery Centre, Lahore, Pakistan from April 2017 to August 2017. Gram-negative organisms, recovered from different specimens of hospitalized and follow-up burn patients resistant to all routinely used antimicrobial drug groups were included in the study. Colistin Minimum Inhibitory concentrations (MICs) were performed by E test for selected organisms.

Results: A total of 434 extremely-drug resistant Gram-negative bacteria, consisting of 244 *Pseudomonas spp* 126 *Acinetobacter spp,* 57 *Klebsiella spp* and 7 *Escherichia spp* were isolated during the present study. Among these Gram-negative bacteria, three Colistin resistant organisms were isolated. All three were non-fermenters. Two isolates were *Pseudomonas aeruginosa* and one was *Acinetobacter baumnnii*. The MIC results of Colistin as tested by E strip were 8 mg/ L for one *Pseudomonas aeruginosa*, 16 mg/L for the other *Pseudomonas* isolate and 12 mg/L for *Acinetobacter species*.

Conclusion: Our study highlights the emergence of Colistin resistance in non-fermenters. This is alarming, as it leaves almost no options for clinicians to treat infections caused by such organisms. It is the need of the hour to establish a policy for antimicrobial stewardship and to control antimicrobial resistance in our country.

Key words: Non-fermenters, Colistin, Carbapenem resistance.

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INTRODUCTION

The emergence of multidrug resistant Gram-negative bacterial infections has become a global health concern [1]. Gram-negative rods including fermenters and non-fermenters are not only resistant to multiple antibiotics but are also becoming resistant to colistin [2,3].

Colistin also known as polymyxin E, is a bactericidal antimicrobial that acts by disrupting the phospholipid structure of the bacterial cell membrane. [4] Colistin was first introduced in the 1950s, but owing to systemic toxicity, the use of colistin was limited to topical treatment only. However, 60 years later, it has become the last-line antibiotic to treat infections caused by carbapenem-resistant *Pseudomonas aeruginosa, Acinetobacter baumanii* and *Klebsiella* species [5]. Colistin resistance has been reported from almost all the continents of the world. Colistin resistance was highest among Asian countries followed by Europe [6].

Guidelines for interpretation of zone size for colistin in Enterobacteriaceae and Acinetobacter species were not defined by the British Society of Antimicrobial Chemotherapy (BSAC), the European Committee on Antimicrobial susceptibility Testing (EUCAST) as well as the Clinical Laboratory Standard Institute (CLSI) [7]. CLSI interpreted the zone size for Pseudomonas aeruginosa only [7,8,9]. The MIC method is the only reliable method to interpret and report colistin susceptibility. For Enterobcateriaceae and *Acinetobacter,* the minimum inhibitory concentration (MIC) breakpoint of ≤ 2 mg/L is interpreted to be sensitive by CLSI, EUCAST and BSAC. Pseudomonas aeruginosa is interpreted to be sensitive at ≤4mg/L by BSAC and EUCAST and ≤ 2 mg/L by CLSI [8,9,10].

Currently, in our country the data on colistin resistance is scarce. We undertook this study to address the issue of colistin resistance among fermenter and non-fermenter Gram-negative rods.

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MATERIALS AND METHODS

This was a descriptive study, carried out in the Microbiology section of Pathology Laboratory of Jinnah Burn and Reconstructive Surgery Centre, Lahore, Pakistan from April 2017 to August 2017. Inoculation of the specimens were carried out on blood agar plates and MacConkey agar plates followed by incubation at 37 °C for 24 hours. Initial and primary identification was performed by Gram staining and oxidase test. The isolates were confirmed using API identification system. Antimicrobial susceptibility testing, as per modified Kirby-Bauer method was performed on all isolates. Interpretation was done in accordance with CLSI 2017. For Quality Control Escherichia coli ATTC 25922 was used.

Gram-negative organisms, recovered from different specimens of hospitalized and follow-up burn patients, that were found to be resistant to all routinely tested antimicrobial drug groups for susceptibility including Penicillins, Cephalosporins, Macrolides, Aminoglycosides, Carbapenems, Fluoroquinolones, Tetracyclines and those Gram-negative organisms which do not possess intrinsic resistance to Colistin were included in this study. We excluded duplicate isolates from a single patient form our study.

The susceptibility to Colistin in *Pseudomonas* species was determined by disk diffusion method using 10 µg Colistin disk (Oxoid Ltd) on Mueller Hinton agar plates. For each strain, a bacterial suspension adjusted to 0.5 McFarland turbidity standards was used. The plates were incubated for 24 hours at 37˚C. Results were interpreted according to CLSI criteria (CLSI 2017). In case of resistance to colistin on disc diffusion method, Colistin E-test strips (BioMerieux) over the range of 0.06 -1024 mg/L were used for validation of Colistin MICs in *Pseudomonas* spp. Determination of MICs of Colistin for all other selected organisms was carried out by applying Colistin E-strip in first place. Suspension of isolated colonies to be tested were made in sterile saline with density adjusted at 0.5 McFarland standard. Inoculum was swabbed on Muller Hinton Plate (Oxoid, UK) followed by application of E-strip on dried agar plates. The plates were incubated at 37°C for 24 hours. MIC showing complete inhibition of growth was recorded.

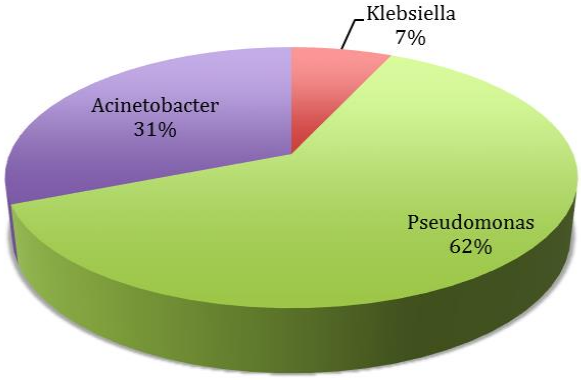
RESULTS

During the present study period (April-August 2017), 244 Pseudomonas species, 126 Acinetobacter species, 57 Klebsiella species and 7 Escherichia species were isolated. Among these 434 Gram-negative bacteria in total, 156 Pseudomonas, 78 Acinetobacter and 17 Klebsiella species were resistant to all the available drug groups including Penicillin, Cephalosporins, Cephems, Monobactams, Carbapenems, Monobactams, Aminoglycosides, Fluoroquinolones, Folate pathway inhibitors and Tetracyclines when tested according to CLSI guidelines (Figure-1).

Table-1: Frequency of Colistin resistance among XDR Gram-negative rods (n=251)

Bacterial Isolate	No of Isolates	Resistance to Colistin	
		No	%
Pseudomonas spp	156	02	1.28
Acinetobacter spp	78	01	1.28
Klebsiella spp	17	00	00
Total	251	03	1.2

Figure-1: Figure 1: Distribution of XDR isolates among Gram-negative rods (n=434).



DISCUSSION

The timely administration of antibiotics can make difference between cure and death for a patient suffering form infection. Unfortunately, years of unrestricted use, misaligned perceptions and limitations in diagnosis have led to the emergence of resistant bacteria as a result of which we are on the brink of a post-antibiotic era [10].

Infections caused by multidrug resistant (MDR) and extensively drug resistant (XDR) Gram-negative bacteria are becoming a global threat to critically ill patients [11].

The terms MDR (Multidrug resistant), XDR (Extensively drug resistant) and PDR (Pan drug resistant) have been well defined by European center of Disease control (ECDC) and Centre for Disease control (CDC), Atlanta. MDR is defined as resistance acquired to at least one drug in three or more antimicrobial categories. XDR is defined as resistance acquired to at least one drug in all

antimicrobial categories, except two or less. While PDR is defined as resistance to all drugs in all antimicrobial categories [12].

The emergence of carbapenem-resistant organisms has greatly limited the treatment options and has led to the increased usage of Colistin in such cases [13]. Currently, data on colistin resistance is lacking in our country. Therefore, it was the need of the hour to determine the extent of this problem in our setup. The frequency of Colistin resistance in our study was 1.2%. A recent study in Pakistan reported colistin resistance to be 0.33 %. [14]. There have been reports of colistin resistance form Saudi Arabia and India as well in the last few years [15, 16].

Colistin resistant Acinetobacter baumanii have also been reported from Asia, Europe, North America and South America [2]. In our study, three colistin resistant organisms were isolated. Two were *Pseudomonas* and one was *Acinetobacte*r species. All these three isolates were found to be extremely drug resistant. Both *Pseudomonas aeruginosa* and *Acinetobacter baumanii* are primarily hospital acquired bacteria and have a diverse array of resistance mechanisms that might steer towards multidrug resistant or even pan drug resistant strains [17].

All three colistin resistant isolates in our study were isolated from burn patients who had severe sepsis and unfortunately died despite aggressive antibiotic therapy. *Acinetobacter baumanii* and *Pseudomonas aeruginosa* are a predominant cause of morbidity and mortality among hospitalized burn patients [18].

Antibiotic selection pressure is a consistent phenomenon in the hospital environment, thus leading to development of antimicrobial resistance. The likelihood of resistance also increases with monotherapy with colistin alone. There is also a possibility that increased use of colistin will increase the incidence of colistin resistance in the near future [19].

On the basis of different studies conducted throughout the world, it is apparent that the emergence of MDR P*seudomonas* and *Acinetobacter* strains is increasing globally [20,21].

It is highly recommended to use colistin in combination with Rifampin, Carbapenem and Tigecycline to decrease the emergence of colistin resistance.

CONCLUSION

The resistance rates to colistin vary globally primarily due to diverse treatment strategies. It is vital to obtain information regarding Colistin resistance as this can help to make guidelines for proper use of this antibiotic. This study was the first attempt to document Colistin resistance in a tertiary care hospital of Lahore. More studies at different institutions of Pakistan are required to know the exact extent of Colistin resistance so that a better infection control policy can be implemented. This will exert a positive effect in reducing morbidity and mortality in patients infected with MDR and XDR bacteria.

AUTHORS CONTRIBUTION

Amina Asif: Planned research work, sample collection, analysis and writeup.

Kanwal Hassan Cheema: Literature review, write up and critical analysis.

Zohaib Ashraf: Literature review

Muna Malik: Literature review

Fareeha Imran: Literature review

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