IN-VITRO ACTIVITY OF COLISTIN IN COMPARISON WITH OTHER ANTIBIOTICS AGAINST GRAM NEGATIVE BACTERIA

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Y.F.Z and S.H. designed the model and the computational framework and analyzed the data. S.S.M. and S.A. carried out the implementation. R.S. performed the calculations. M.A.K. and S.M. wrote the manuscript with input from all authors. Y.F.Z and S.M. conceived the study and were in charge of overall direction and planning.

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ABSTRACT:

Background:

Majority of gram negative bacterias have developed resistance and are multidrug resistant which increased the mortality rate. In the current era when there is higher rate of multidrug resistance there is a need to review all the antibiotics which were previously used. Recent literature found a promising effect of colistin against multidrug resistant so the current study aimed to find out the in vitro activity of colistin in comparison with other antibiotics against gram negative bacteria.

Methods:

A Quasi experimental study was conducted in department of Pharmacology, Ziauddin University. Sample from pus, wound swab, blood, urine and from endotracheal secretion were collected. Samples were cultured on Blood agar and MacConkey Agar. Antibiotic susceptibility testing done by Kirby Bauer Disc diffusion technique using Mueller Hinton Agar plate. Agar dilution method was used to find the minimum inhibitory concentration (MIC). Data was analyzed by using Statistical Package for Social Sciences (SPSS) version 21. P value of less than 0.05 was considered as significant.

Results:

The mean age of study participants was 54 ± 20 years. About 176 strains of gram negative bacteria were tested, out of which 79 strains were multidrug resistant while 97 strains were of non-multidrug resistant gram negative bacteria. Colistin showed good in-vitro activity against many strains of the multidrug resistant gram negative bacilli as it was 100% sensitive against Acinetobacter and E.coli with zero resistance. Colistin was also very effective against pseudomonas aeruginosa and klebsiella pneumonia with sensitivity of 99% and 94% respectively. Only the enterobacter, among all MDR strains showed 75% sensitivity with 25% resistance.

Conclusion:

It can be concluded that colistin is highly sensitive against multidrug resistant gram negative bacilli including Acinetobacter, E.coli, Pseudomonas aeruginosa and klebsiella pneumonia with little or no resistance in comparison to other antibiotics.

Keywords: Gram negative bacilli, Multidrug resistant, Colistin

INTRODUCTION:

Antimicrobials agents are main therapeutic tool in medicine to treat variety of infections caused by bacteria. The development of antibiotics is considered as one of the most important advances of modern science. Antibiotics have saved millions of lives. Emergence of resistance against antimicrobials is one the most important threats globally (1). According to the World Health Organization (WHO), antibiotic resistance has now become a major clinical issue and global public health problem in this century (2). In developing countries, physicians prescribed antibacterial drugs without performing diagnostic susceptibility test. It has been reported that

clinicians prescribed broad spectrum antibiotics in circumstances where more appropriate or specific narrow spectrum antibiotics can be used (3). One of the important factors of antibiotic resistance is irrational use of antibiotics which is seen in many developing countries where most of the drugs can be purchased over the counter without any advice or prescription of the physician (4). One of the other reasons of antibiotic resistance is the patient's incompliance for recommended treatment; either patient stop taking antibiotic drugs due to any reason or forget to take medications (5).

Majority of gram negative bacterias have developed resistance and are multidrug resistant which increased the mortality rate. Literature revealed that nosocomial (hospital acquired) infections are mostly because of gram negative bacteria in which majority are multidrug resistant so leads to prolonged hospital stay i.e. more than 5 days, resulting in increased morbidity rate and hospital cost (6, 7). Gram negative bacteria has the capability of upregulate or down regulate the gene expression or even acquire new gene encoding, sometimes cause mutation or horizontal gene transfer, resulting in drug resistance by enzyme down regulation like in extended spectrum β-lactamase or carbapenemase (8). World Health Organization (WHO) identified few gram negative bacteria and placed them in the priority list of Critical category, including carbapenem resistant strain like Acinetobacter baumannii and Pseudomonas aeruginosa and 3rd generation cephalosporin resistant strains like E.coli, Enterobacter and Klebsiella pneumonia (9).

Colistin was first discovered in 1947 and clinically used in 1958 but the use was restricted in early 1970 because of its neurotoxic and nephrotoxic effects (10, 11). In the current era when there is higher rate of multidrug resistance there is a need to review all the antibiotics which were previously used (12, 13). Recent literature found a promising effect of colistin against multidrug resistant P. aeruginosa and Acinetobacter baumanii in ventilator associated pneumonia and Klebsiella pneumonia in bacteremia. Studies conducted in critically ill patients reported that intravenous therapy is highly effective in the treatment of bacteremia, pneumonia and urinary tract infections with less toxic effect as compared to previously reported (14). Studies have been conducted on the chemistry, antimicrobial activity, mechanism of action, pharmacokinetics, pharmacodynamics, adverse effects and resistance pattern against colistin (15-17) so the current study aimed to find out the in vitro activity of colistin in comparison with other antibiotics against gram negative bacteria.

MATERIAL AND METHODS:

A Quasi experimental study was conducted in department of Pharmacology, Ziauddin University and the samples were collected from Ziauddin hospital, Nazimabad. Study got ethical approval from the concerned institute. Sample from pus, wound swab, blood, urine and from endotracheal secretion were collected following guidelines of microbiology lab of Ziauddin hospital, Nazimabad from October 2021 to April 2022. Samples from all age groups were included in the study while those samples which having either double growth or contamination on agar plates were excluded.

About 5ml of blood was inoculated in BACTEC blood culture bottle having a rich growth media. It was placed in BACTEC blood culture system and incubated further for five days. After

scanning the bar code samples were cultured on Blood agar and MacConkey Agar. Blood agar was used to isolate hemolytic bacteria like streptococcus and MacConkey agar is selective for gram negative bacteria. Gram staining was done on bacterial growth and gram negative rods were processed for biochemical identification and antibiotic sensitivity.

Antibiotic susceptibility testing by Kirby Bauer Disc diffusion technique This is one of the most commonly employed methods for antibiotic susceptibility testing. In this method a lawn of bacterial inoculum was made on 150 mm Mueller Hinton Agar plate. Commercially prepared fixed concentration paper antibiotic discs were placed on agar plate. Before determination of results, plates were incubated for 16-24 h at 35 ºC. The zones of growth inhibition around each of the antibiotic disc were measured in accordance to CLSI guidelines (2018) and labeled as either sensitive or resistant (18). Antibiotic susceptibility was tested for amikacin, ampicillin-sulbactam, ceftazidime, ciprofloxacin, Imipenem, gentamicin, piperacillin-tazobactam and trimethoprim-sulphamethoxazole. Agar dilution method was used to find the minimum inhibitory concentration (MIC) of colistin as per CLSI guidelines (19). Gram negative bacilli stains having MIC more than 4mg/dl were labelled as resistant.

Data was analyzed by using Statistical Package for Social Sciences (SPSS) version 21. Frequencies and percentages were calculated for categorical variables. Age group and specimen distribution were mentioned in terms of frequency and percentages. Chi square test was applied to find out the association between age groups and the presence of multidrug resistance. P value of less than 0.05 was considered as significant. Multiple bar charts were used to depict the susceptibility pattern of colistin with different gram negative bacilli.

RESULTS:

The mean age of study participants was 54 ± 20 years. Majority of the cases of gram negative bacillary infection were obtained from age group 61-80 year, having both MDR and non-MDR strains of gram negative bacilli and very few were obtained from the age group above 81 years as mentioned in Table 1. Age has no association with the risk of having infection with MDR or non-MDR strains of gram negative bacteria as the p-value was non-significant (p-value ≥ 0.05).

Table 1 Frequency of MDR strains of gram negative bacteria in different age groups

Age groups	MDR strains	Non-MDR strains	p-value
1 month - 20 years	4 (5.1%)	4 (4.1%)	0.38
21- 40 years	23 (23.7%)	28 (35.4%)	
41- 60 years	22 (22.7%)	19 (24.1%)	
61- 80 years	28 (35.4%)	46 (47.4%)	
≥ 81 years	2 (2.1%)	--	

About 176 strains of gram negative bacteria were tested, out of which 79 strains were multidrug resistant while 97 strains were of non-multidrug resistant gram negative bacteria. The most common multidrug resistant gram negative bacilli were tested with different antibiotics to check the susceptibility pattern as shown in Table 2.

Table 2 Susceptibility pattern of MDR strains of gram negative bacteria									
		AM	SAM	CAZ	CIP	IMP	GEN	TZP	SXT
Acinetobacter	n=16	12	10	8	5	6	7	5	7
	%	75	62	50	31	37	44	31	44
Enterobacter	n=8	7	--	4	4	7	5	4	8
	%	88		50	50	88	63	50	50
E.coli	n=11	9	--	4	3	11	8	7	4
	%	82		36	27	100	73	64	36
Klebsiella	n=15	6	--	3	2	13	4	2	2
	%	40		20	13	87	27	13	13
P. aeruginosa	n=29	14	--	8	8	12	7	14	--
	%	48		28	28	41	24	48	

AN: amikacin; SAM: ampicillin-sulbactam; CAZ: ceftazidime; CIP: ciprofloxacin; IMP: imipenem; GEN: gentamicin; TZP: piperacillin-tazobactam; SXT: trimethoprim-sulphamethoxazole

Colistin showed good in-vitro activity against many strains of the multidrug resistant gram negative bacilli as it was 100% sensitive against Acinetobacter and E.coli with zero resistance. Colistin was also very effective against pseudomonas aeruginosa and klebsiella pneumonia with sensitivity of 99% and 94% respectively. Only the enterobacter, among all MDR strains showed 75% sensitivity with 25% resistance as presented in Figure 1.

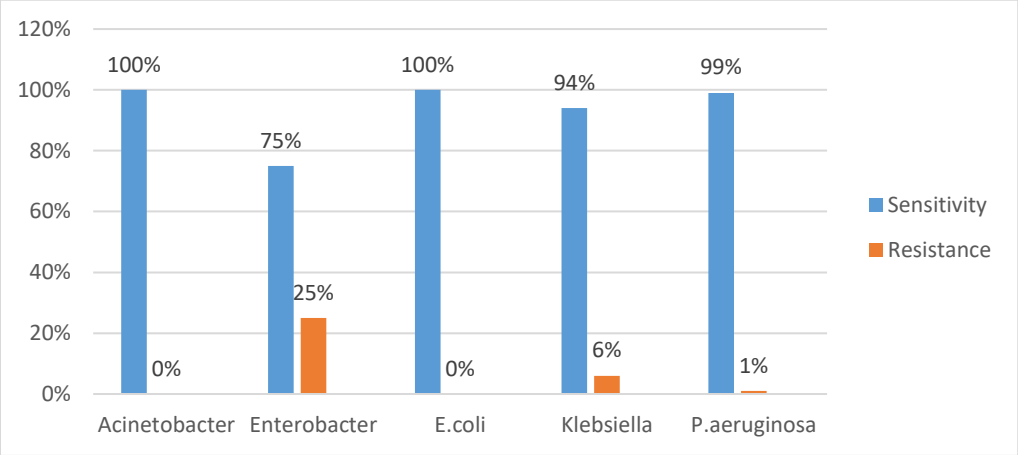

Figure 1 In-vitro susceptibility of colistin against MDR strains of gram negative bacilli

DISCUSSION:

Colistin has a bactericidal effect as it attacks on the cell wall of the bacteria by disturbing ion channel stability specially of magnesium and calcium resulting in increased cell permeability and ultimately cell death (20). Literature revealed that colistin is highly sensitive against Acinetobacter, E.coli, pseudomonas aeruginosa and klebsiella pneumonia with little or no resistance but it shows no response against cocci including both gram negative and gram positive cocci, anaerobes and gram positive bacilli (21). Literature review revealed that in the cases of P. aeruginosa. the resistance develops because of mutated OprH protein found in the cell membrane

but in in-vitro studies there is sometimes adaptive resistance developed by alterin lipopolysaccharide fatty acid composition. In Salmonella infection resistance develop as a result of change in negatively charged lipopolysaccharides (22).

Currently, it's the era of multi drug resistant infection because of either irrational use or misuse of antibiotics or poor compliance from patient side. Majority of gram negative bacilli are multidrug resistant speciaaly against beta-lactamases, aminoglycosides and the carbapenems (5). In such scenario the colistin reported miraculous effect in very critical conditions. It acts like a life saver in serious cases of bacteremia, pneumonia specially ventilator associated pneumonia and severe urinary tract infections with less toxic side effects in comparison to other available antibiotics. Because of its life saving effect it is highly used through parenteral route in critically ill patients so there is a need to up to date the susceptibility pattern of colistin in different geographical regions among different ethnic population (23).

Colistin susceptibility testing remains a challenge because of lack of standrized disc method for checking susceptibility. Some of the studies found that disc method is inaccurate and gives false results and recommended agar dilution method or micro-dilution methods. In the cases of Acinetobacter the E-test method is accurate to check colistin susceptibility (24).

Literature review revealed variable results about the susceptibility pattern of colistin among different regions. A study conducted incystic fibrosis patients of United Stated reported 31% resistance of colistin against P. aeruginosa and E-test was used for susceptibility testing and susceptibility breakpoint was 4mg/dl (25). Anothr study from the same region used agar dilution method with susceptibility breakpoint of 4mg/dl and found same resistance rate against P. aeruginosa, beside that also found 32% resistance rate against Enterobacter and 12% against klebsiella. A study conducted in other region also used agar dilution method at susceptibility breakpoint of 2mg/dl and found lower level of colistin resistance against Acinetobacter and Enterobacter species (26). Current study found 100% sensitivity of colistin against Enterobacter while only 1% cases reported resistance against P. aeruginosa. This variation in the susceptibility pattern of colistin confirmed the importance of geographical location and ethnicity, so area and ethnic based studies should be done to confirm the susceptibility pattern of colistin.

CONCLUSION:

It can be concluded that colistin is highly sensitive against multidrug resistant gram negative bacilli including Acinetobacter, E.coli, Pseudomonas aeruginosa and klebsiella pneumonia with little or no resistance in comparison to other antibiotics.

CONFLICTS OF INTEREST:

The authors reflect no conflict of interest.

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