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Colistin Resistance among Gram Negative Organisms; an Evolving Problem from Tertiary Care Hospital, Pakistan 2014

Tuba Bashir and Altaf Ahmed

Department of Microbiology, The Indus Hospital, Korangi Crossing, Karachi, Pakistan

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Corresponding Author:
Tuba Bashir
Department of Microbiology,
The Indus Hospital, Korangi Crossing, Karachi, Pakistan
Off: +92-21-35112709-17;
Fax: +92-21-35112718
Email: tuba_bashir@yahoo.com;
tuba.bashir@tih.org.pk

Abstract: This Study was design to report the evolving Colistin Resistant Gram Negative Organisms from Pakistan in 2014. From 885 isolates of hospitalized patients, 03 Colistin resistant isolates were reported, 02 were *Escherichia coli* and 01 was *Acinetobacter specie*. Colistin of all three isolates was tested by Disk diffusion method, MIC by Phoenix 100 (BD) and E-strip. This report contributes a useful addition in literature of Gram Negative Organisms Susceptibility pattern reported from Pakistan, as Colistin is extensively used for the treatment of Multi drug resistant Gram Negative's in hospital settings.

Keywords: Colistin Resistance, Gram Negative Organisms, Pakistan

Introduction

Colistin is bactericidal antibiotic; it binds to lipo polysaccharides and phospholipids of Gram Negative Bacterial Cell membrane. This binding results the intracellular leakage of Cell components and finally cell death (Alfahad and Omrani, 2014). It was introduced in 1952 in clinical practice to treat infections caused by Gram Negative Bacteria (Biswas *et al*., 2012). In 1970's better antimicrobials was available to treat Gram Negative Infections, as Colistin has poor safety profile (Alfahad and Omrani, 2014).

The Guidelines for Interpreting colistin zone size of *Enterobacteriaceae* and *Acinetobacter specie* were not defined by Clinical Laboratory Standard Institute (CLSI), The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and The British Society for Antimicrobial Chemotherapy (BSAC, 2014). CLSI (2014) interpreted the zone diameter for *Pseudomonas aeroginosa* only (CLSI, EUCAST and BSAC, 2014).

The only reliable Method for Interpreting and reporting of Colistin is MIC method. For *Enterobacteriacea* and *Acinetobacter*, the MIC break point of ≤ 2 mg L^{-1} interpretated as Sensitive by CLSI, EUCAST and BSAC. *Peudomonas aeroginosa* interpretated as sensitive at ≤ 2 mg L^{-1} by CLSI and $\leq$ 4mg L^{-1} by EUCAST and BSAC (CLSI, EUCAST, BSAC, 2014).

This Study was done in the department of Microbiology, The Indus Hospital, Korangi Crossing, Karachi, Pakistan. For this Study, the data was analyzed from January 2014 to December 2014.

All Gram Negative Organism which were not intrinsically resistant to Colistin were included in this study. Specimen from wound, pus, fluids, urine, respiratory secretions etc were included:

- Duplicate isolates of the same patient were excluded in this study
- Initially, All Isolates were processed and identified by Standard Conventional method
- For Quality Control *Escherichia* coli 25922 were used

Colistin Resistant Isolates by Conventional methods were also tested on Phoenix 100 (Becton Dickinson, San Diego, USA). NMIC/ID-94 panels were used for identification and MIC testing of Isolate. Quality Control testing was also performed according to manufacturer's instruction.

Colistin E-test strips (bioMeieux) ranging from 0.06-1024 mg L^{-1} was also used for further verification of Colistin resistance. Colonies of the tested Isolate were suspended in sterile saline, density of the inoculum were adjusted at 0.5 McFarland standard, Swabbing of inoculum on Muller Hinton Plate (OXOID, UK). E-strip was applied after drying agar surface. Incubate the plates at 37°C for 18-24 h.

In 2014, we reported 476 *Escherichia coli*, 157 *Klebsiella specie*, 152 *Pseudomonas aeroginosa* and 100

Acinetobacter specie in hospitalized patients. Among these 885 Gram Negative Organisms we Isolated 03 Colistin resistant Organisms. 02 were *Escherichia coli* and 01 was *Acinetobacter specie*.

The Colistin MIC of all three isolates was reported as > 4 mg L^{-1} by Automated Phoenix 100. The Tigecycline MIC was ≤1 mg L^{-1} for both *Escherichia coli* and 2 mg L^{-1} for *Acinetobacter specie* by Phoenix 100.

The E-strip MIC results of Colistin were 8 mg L for both *Escherichia coli* and 16 mg L^{-1} for *Acinetobacter specie*.

We did not find any *in vitro* Colistin resistance in *Klebsiella specie* and *Pseudomonas aeroginosa.*

The best of our knowledge, this is the 1st report of Colistin resistance among Gram Negative Organisms from Pakistan (Pubmed). Reports of *in vitro* Colistin resistance has been published from Saudi Arabia and India in recent years (Musa *et al*., 2013; Abeer *et al*., 2013) From Greece *in vitro* colistin resistance was also reported (George *et al*., 2010). Colistin has been extensively used to treat Multi drug resistant Gram Negative infections (Alfahad and Omrani, 2014).

It has been reported that the growing threat of Colistin resistance might be due to its extensive use as a result of increased Carbapenem resistance among Gram Negative Organisms (Musa *et al*., 2013).

For clinical laboratories, colistin reporting by disk diffusion method is not recommended, as for *Enterobacteriaceae* and *Acinetobacter spp*. no interpretation criteria is available by CLSI. Despite the guidelines criteria MIC testing should also encourage in MDR isolates to improve treatment strategies, leads to better outcomes.

In our study, Table 1 showed that 03 patients were reported as colistin resistant isolates, 02 were *Escherichia coli* and 01 was *Acinetobacter specie*. 02 were male patients and 01 was female. All Colistin resistant isolates were sensitive to Tigecycline. 02 were Sensitive only to Tigecycline and Tetracycline whereas, 01 Colistin Resistant Isolate was Sensitive to many other antibiotics including carbapenems. This finding wass also reported in other publications (George *et al*., 2010). Colistin MIC of all three isolates was very high done by E-strip (Fig. 1).

The limitation of this study was that; we did not perform molecular testing; therefore we are not able to report that which genes are responsible for the Colistin resistance.

On the other hand, there is a great need of MIC reporting of Colistin due to extremely high resistance among Gram Negative's and extensive use of Colistin in hospitalized patients.

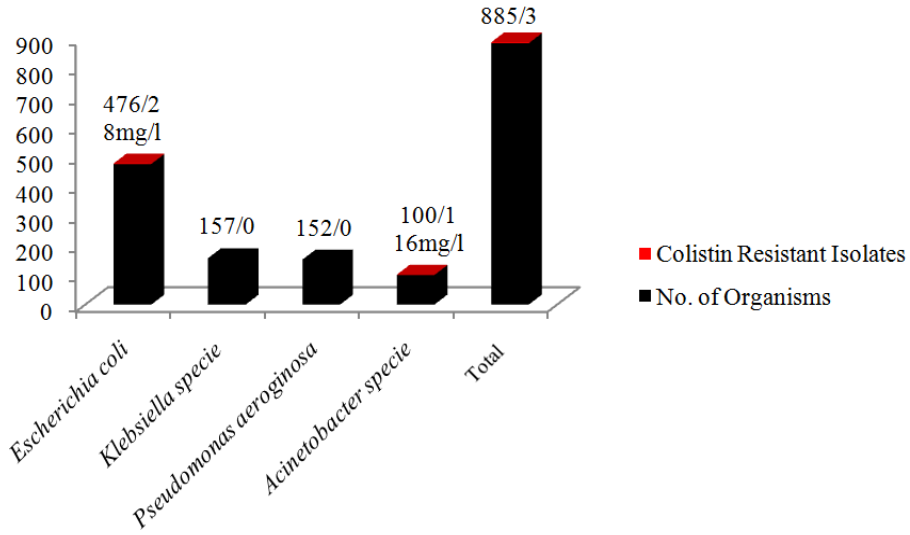

Fig. 1. No. of Colistin Resistant Isolates with MIC by E strip (mg/L) from Total Carbapenem Resistant Isolates in In-patients for the year 2014

Table 1. MIC profile of Colistin resistant isolates by phoenix 100 (mg L^{-1})

Isolate/Sex	Age	Sample	AMP	AMC	TZP	CIP	CN	AK	IPM	MEM	TGC	SXT	CT
Acinetobacter spp.	44/F	Urine CS	>16	>16/8	>64/4	>2	>8	>32	8	>8	2	>4/76	>4
Escherichia coli	25/M	Urine CS	>16	>16/8	>64/4	>2	>8	>32	8	>8	≤1	>4/76	>4
Escherichia coli	19/M	Wound CS	>16	>16/8	≤4/4	>2	>8	≤8	≤1	≤1	≤1	>4/76	>4

Abbreviations for Antibiotics: Amp = Ampicillin; AMC = Amoxicillin-clavulanate; CIP = Ciprofloxacin; AK = Amikacin; CN = Gentamicin; SXT = Trimethoprim-Sulfamethoxazole; TZP = Piperacillin/tazobactam; IPM = Imipenem; MEM = Meropenem; TGC = Tigecycline; CL = Colistin

Conclusion

This report is unique, as it is the 1st *in vitro* Colistin resistance report from Pakistan. Colistin resistance is an alarming concern because it is used as last resort of treatment in healthcare facilities. Therefore, Strict infection Control guidelines and Antimicrobial Stewardship should implement to overcome the new resistance spread and MIC reporting of MDR isolates should encourage.

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Author's Contributions

Both Authors Contributed equally to the Article.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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