ORIGINAL ARTICLE

# *IN VITRO* SUSCEPTIBILITY PATTERN OF EXTENDED SPECTRUM B-LACTAMASE PRODUCING GRAM NEGATIVE BACILLI AGAINST TETRACYCLINES

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**Background:** Extended Spectrum β-lactamases (ESBLs) are emerging as common nosocomial pathogens and important cause of mortality and morbidity, if not treated properly. The need of the hour is to find effective treatment options for dealing with ESBL producing organisms. This study was aimed to evaluate *in vitro* susceptibility pattern of extended spectrum β-lactamase producers against tetracyclines. **Methods:** This descriptive cross-sectional study was carried out in the department of Microbiology, Army Medical College, Rawalpindi, National University of Sciences and Technology over a period of 6 months. Seventy eight non-duplicate isolates were included in the study. ESBL detection was done using Jarlier *et al* method. *In vitro* susceptibility of tetracyclines like tetracycline, doxycycline, minocycline and tigecycline was then tested using Modified Kirby Bauer disc diffusion method. The zones of inhibition were measured after completion of incubation period and interpreted as per CLSI and FDA guidelines. **Results:** Approximately 56.4% of the isolates were *Escherichia coli,* 28.2% were *Klebsiella pneumoniae*, 10.26% were *Enterobacter species,* and 2.6% were each *Klebsiella oxytoca* and *Acinetobacter species*. ESBLs were found to be most sensitive to tigecycline, intermediate in susceptibility to minocycline while least sensitive to doxycycline and tetracycline. **Conclusion:** Among tetracyclines, tigecycline has best *in vitro* susceptibility against ESBL producing Gram negative rods.

**Keywords:** Extended spectrum β-lactamases (ESBLs), tetracyclines, susceptibility

**J Ayub Med Coll Abbottabad 2015;27(4):788–90**

## INTRODUCTION

The Extended Spectrum β-Lactamases (ESBLs), which belong to the Bush Jacoby Medeiros class 2be, are the enzymes which confer resistance to all penicillins, monobactams, and cephalosporins (excluding cephamycins).[1] ESBLs were first described in 1983 and have a frequency of 35.5–45% among various clinical specimens.[2–4] They are produced by both enterobacteriaceae and the non-fermenting Gram negative organisms like Acinetobacter and *Pseudomonas* spp.[5]

Gram negative organisms possessing ESBL are a serious threat to hospitalized patients because they hydrolyze extended spectrum cephalosporins, which are often used in the management of hospital-acquired infections.[2] Treatment failure may result if patients suffering from infections caused by ESBL-producing organisms are managed with aztreonam or extended-spectrum cephalosporins despite *in vitro* susceptibility of causative organisms to these antibiotics by routine susceptibility testing. [2]

Tetracyclines, like tetracycline, doxycycline, minocycline and tigecycline are a group of bacteriostatic antibiotics discovered in 1945.[6] They act by inhibition of the protein synthesis at the translation phase, by inhibiting the binding of aminoacyl t-RNA at the 30S ribosomal subunit binding site of the bacteria.[7] They had been used for many years against various infections however their activity was limited by the emergence of resistance due to efflux pump and the ribosomal binding. [8] Tigecycline is a new, broad spectrum, long acting, 9-tert butyl glycylamide derivative of minocycline which has an added potential against the bacterial resistance by bypassing the efflux pump and by binding avidly to the ribosomal binding site of bacteria.[7] Thus the drug can accumulate inside the bacteria and is retained for a longer time at its site of action.ou[7] Tigecycline has been found to be effective against many Gram negative and Gram positive organisms including the multi-drug resistant ones.[9]

ESBL dissemination occurs through spread via mobile genetic elements between strains of different as well as same species. Sometimes there is an associated co-resistance of the tetracyclines with other antibiotic groups like quinolones, chloramphenicol, aminoglycosides, sulphonamides and trimethoprim. This not only limits our treatment options but is also responsible for the persistence and spread of the isolates. In such situations, alternative antibiotics are required

to treat these infections.[10] A study to evaluate the efficacy of different tetracyclines against ESBL producing Gram negative bacilli is the need of the hour and has never been conducted in our set up before. This study was aimed to find out the susceptibility pattern of the ESBL producing Gram-negative bacilli against different tetracyclines.

## MATERIAL AND METHOD

This descriptive cross-sectional study was carried out in the department of Microbiology, Army Medical College, Rawalpindi, from October 2009 to March 2010. Seventy-eight, non-duplicate clinical specimens were included in the study. These specimens were subjected to standard microbiological procedures.

The species level identification of the organisms was done by Analytical Profile Index (API)-20E. The ESBL detection was done by the double disc synergy method recommended by Jarlier *et al* (Figure-1). The susceptibility testing of tetracyclines was done by Kirby Bauer Disc Diffusion method.

A 0.5 McFarland turbidity standard bacterial suspension was prepared for each isolate and inoculated on the Mueller Hinton Agar plate. This was followed by application of antibiotic discs (Oxoid) of tetracycline (30µg), doxycycline (30µg), minocycline (30µg) and tigecycline (15µg).

These plates were then incubated for a period of 18–24 hours. at 37 $^{o}$C. The results of the zones of inhibition of tetracycline, doxycycline and minocycline were interpreted as per Clinical and Laboratory Standard Institute (CLSI) guidelines and the results of the tigecycline were interpreted according to the Food and Drug Authority (FDA) recommendations.[11,12]

## RESULTS

Out of a total of 78 ESBLs, 42.5% of the ESBL producing bacteria were isolated from the urine specimen, 19.1% from pus, 14.8% from double lumen tip, 8.5% from high vaginal swabs (HVS), 6.3% from blood, 4.2% from sputum and 2% from each tissue and fluid.

Among these ESBL producing bacteria, *Escherichia coli* were the most commonly isolated (56.4%) while *Acinetobacter baumannii* and *Klebsiella oxytoca* were least commonly isolated (2.6%) (Table-1). A comparison of tetracyclines against the ESBL producing Gram-negative bacilli revealed tigecycline to be most effective with 96% susceptibility of ESBLs against it, minocycline was intermediate (68%) in susceptibility. Eight percent of the ESBL producers were susceptible to doxycycline and 6% to tetracycline.

**Figure-1: Key hole zone in Jarlier *et al* method**

**Table-1: Percentages of different Gram-negative rods among ESBL producers (n=78)**

| ESBL isolate | Number | Percentage (%) |
|---|---|---|
| *Escherichia coli* | 44 | 56.4% |
| *Klebsiella pneumoniae* | 22 | 28.2% |
| *Enterobacter species* | 8 | 10.26% |
| *Acinetobacter baumannii* | 2 | 2.6% |
| *Klebsiella oxytoca* | 2 | 2.6% |

## DISCUSSION

The imprudent use of broad spectrum anti-microbial agents for managing various infections has led to the rapid emergence of ESBL producing Gram negative bacilli. According to CLSI, if new interpretive zone sizes for third generation cephalosporins are followed, separate ESBL detection is not required however ESBL detection may be done for epidemiological and infection control.[11] The high frequency of ESBL producing Gram negative organisms and its potential to cause outbreaks are a matter of great concern for both the patients and the health care providers and warrants the necessity to determine new treatment options.[13]

In our study, ESBLs were found to have a 30% better susceptibility to tigecycline as compared to minocycline. Our results are in coherence with a study conducted in United States of America (USA), in 2004, in which ESBL producing Gram-negative bacilli isolates from Pakistan, India, Australia and Philippines were included. In this study, Johnson *et al* found that the Extended Spectrum β-Lactamase (ESBL) producing Gram-negative bacilli were sensitive to tigecycline in 98% of cases and to minocycline in only 71%.[14] Similarly in a larger scale study, conducted by Hoban et al the results were found to be similar to our results. In their study, ESBL producing *Klebsiella pneumoniae* were found to be 91.3% susceptible to tigecycline and 69.6%

susceptible to minocycline.[15] In the same study, the ESBL producing *Escherichia coli* were found to be 100% susceptible to tigecycline while 66.7% susceptible to minocycline.[15]

In another study conducted in Spain in 2006, Morosini *et al* found that against the ESBL producing bacteria, tigecycline ($MIC_{50}$, 0.5µg/ml; $MIC_{90}$, 1 µg/ml) had upto 256-fold better activity as compared to doxycycline and minocycline. The percentage susceptibility of ESBLs against minocycline, tetracycline, tigecycline and doxycycline, were found to be 69.5%, 38.2%, 97.5% and 45.6% respectively.[10] For minocycline and tigecycline the results of the two studies are comparable however as compared to our results, their isolates showed a higher susceptibility to tetracycline and doxycycline.

The adverse effects of all the tetracyclines are mild on gastrointestinal tract like nausea and vomiting, teeth discoloration in children and vertigo.[7] The gastrointestinal side effects are relatively more common with tigecycline as compared to the other three tetracyclines.[16] Mild haematological abnormalities like thrombocytopenia, neutropenia and eosinophilia can occur rarely with tetracycline, doxycycline and minocycline while no haematological abnormality has been documented with tigecycline.[16] Tetracycline, doxycycline and minocycline can be given both orally and parenterally but tigecycline can only be administered parenterally.[7] As far as the cost of the drugs is concerned, the tetracycline, doxycycline, minocycline are quite economical while tigecycline is an expensive drug. In the setting of infections by multi-drug resistant organisms like ESBL producers, tigecycline remains our most reliable resort among all tetracyclines.

## CONCLUSION

Although minocycline has an ease of oral administration and low cost yet in 30% of infections caused by the Gram negative organisms which produce ESBL, tigecycline is the only solution among all tetracyclines.

## AUTHOR'S CONTRIBUTION

MMG: Conception and Design, Acquisition of data, Drafting work & Re-visiting it. JU: Drafting work and Final approval. AH: Acquisition, analysis and interpretation of data. FK: Acquisition, analysis and interpretation of data. **NS**: Drafting work & Re-visiting it.

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