



## SUSCEPTIBILITY PROFILE OF MULTIDRUG RESISTANT GRAM NEGATIVE BACTERIA TO A NEOADJUVANT ANTIBIOTIC, ELORES

### Microbiology

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### ABSTRACT

**Background:** Novel approaches are required to counter the threat of emerging multidrug resistance Gram negative bacteria (GNB) in clinical settings. Efflux pump inhibitors to overcome drug resistance have been a field of interest for past two decades. In this study we explored the efficacy of recently launched neoadjuvant antibiotic entity, “elores” comprising of ceftriaxone, sulbactam and EDTA, against multidrug resistant Gram negative bacteria.

**Materials and methods:** A total of 150 multidrug resistant GNBs were isolated from various clinical samples were included in study. All the isolates except *P.aeruginosa* and *Burkholderia cepacia* were tested for susceptibility against elores.

**Results:** Out of 136 MDR GNBs tested, 76 (55.88%) were sensitive to elores. Maximum sensitivity was observed for *E.coli* (72.97%) followed by *Klebsiella pneumoniae* (48.33%), *Acinetobacter baumannii* (57.14%).

**Conclusion:** The results shows that significant number of MDR GNB were susceptible to elores and its inclusion in treatment of these infections should be considered.

### KEYWORDS

#### INTRODUCTION

Though the antimicrobials have been in use since ancient times, modern era of antibiotics began with discovery of salvarsan by Paul Ehrlich in 1909. This was followed in quick succession with discovery of sulfonamides and penicillin. Then came the golden era of antibiotics (1950-1970) when newer antibiotics were discovered 1. With the availability of antibiotics for most of the infections, it became a perception that infectious diseases were no more the most important public health issue 2. However, soon the realization dawned that the bacteria were developing resistance to antibiotics faster than the rate at which they were being discovered.

In present scenario, emergence of multi drug resistant (MDR) Gram negative bacteria (GNB) poses a major challenge to successful use of antibiotics for treatment of infectious diseases worldwide. Although these MDR GNB were initially encountered in hospitalized patients, they are now frequently isolated from community acquired infections. This problem of MDR is more often encountered in developing countries where there is more reliance on use of antibiotics due to higher burden of infectious diseases compounded by poor healthcare infrastructure and infection control practices. Analysis of trends in antimicrobial resistance in isolates from blood culture over 10 years (2000 to 2009) published by Datta et al clearly show the alarming rate at which MDR is increasing in Gram negative organisms. Carbapenem resistance increased from 2.4% to 52% in *K. pneumoniae* 3. Another study on a large cohort reported carbapenem resistance in 60% of *K. pneumoniae* isolates 4. Drug resistant strains are now commonly isolated from various samples obtained from community 5-6. This is a very disturbing trend and requires serious introspection by health care workers and policy makers.

Due to lack of better alternatives, research is now focused on discovering unconventional targets and novel groups to develop newer antibacterials. One potential target for antibacterial substances is the efflux pumps in bacterial cell wall. They are fairly well characterized both in Gram positive and Gram negative bacteria. Efflux pump play vital role in bacterial virulence and pathogenicity, including formation of biofilms 7. One of the compounds, Ethylenediamine tetraacetic acid (EDTA) has been introduced in clinical practice as neoadjuvant with

Ceftriaxone and Sulbactam combination (Elores). This combination has shown to down regulates efflux pump AcrAB-TolC mRNA, possibly by chelating calcium ions which are essential for ATP production which is in turn is required for mRNA transcription 8. Also, this disturbance in the ATP production directly affects the efflux pump which is dependent on metabolic energy for its functioning 9. In order to ascertain elores as an additional option to combat MDR isolates in a clinical setting, an in-vitro study was conducted at the microbiology department of a tertiary care hospital. The objectives of the study were to identify MDR Gram negative isolates and test the antimicrobial activity of novel drug combination of ceftriaxone, sulbactam and EDTA (elores) against them.

#### MATERIAL & METHODS

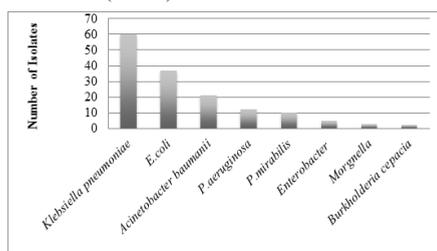
All clinical samples received for bacterial cultures between June to Dec 2017 were included in the study. Blood samples were cultured using fully automated blood culture systems, Bact/Alert 3D (BioMerieux, France) and Bactec 9120 (BD, USA). Other clinical samples were processed using conventional culture media. After obtaining a pure growth, the isolate identification and antibiotic sensitivity testing was performed by Vitek 2 Compact (BioMerieux, France). MDR isolates, defined as those detected to be resistant to penicillins, cephalosporins and carbapenems were included in the study.

Antimicrobial susceptibility against ceftriaxone-sulbactam with EDTA combination (Elores) was performed by disc diffusion method using antibiotic discs from HiMedia, India. Sensitivity of the isolated organisms against this combination were reported as sensitive or resistant based on standards for ceftriaxone as mentioned in Clinical Laboratory Standards Institute guidelines. Isolates of *Paeruginosa* and *Burkholderia cepacia* were excluded as there were no testing standards mentioned in CLSI guidelines against ceftriaxone.

#### RESULTS

A total of 150 MDR Gram negative bacterial isolates were isolated during study period. The distribution of the 150 bacterial isolates is show in Figure 1. A total of 136 out of 150 isolates were further tested for antimicrobial susceptibility against ceftriaxone-sulbactam with

EDTA combination (Elores).



**Figure 1:** Profile of MDR Gram negative isolates from clinical samples They were isolated from various clinical samples from both inpatients and OPD which included blood, urine, pus, semen, bile, tracheal aspirate, bronchial aspirate lavage (BAL), drain fluid, tissue and pleural fluid (Table 1). Maximum isolates were from urine followed by pus and blood, which accounted for almost 85% of the total samples.

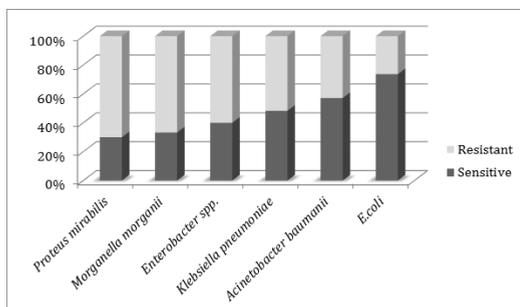
**Table 1: Clinical samples included in the study**

S No	Sample	Number (%)
1.	Urine	75 (50)
2.	Pus	31 (20.7)
3.	Blood	21 (14)
4.	Tracheal aspirate	10 (6.7)
5.	Tissue	05 (3.3)
6.	Semen	02 (1.3)
7.	Bal	02 (1.3)
8.	Pleural fluid	02 (3.3)
9.	Drain	01 (0.7)
10.	Bile	01 (0.7)
Total	150	

Elores displayed significant susceptibility against these pathogens. 76 of 136 MDR isolates were sensitive to ELORES (55.88%). The sensitivity pattern of different MDR isolates towards elores is shown in Table 2 and Figure 2. The susceptibility of the three most common isolates included in our study i.e. *Klebsiella pneumoniae*, *E. coli* and *Acinetobacter baumannii* towards elores was 48.33%, 72.97% and 57.14% respectively.

**Table 2: Sensitivity pattern of various isolates against Elores**

S.No	Organism	No. of Sensitive organisms	% Sensitivity
1.	<i>Klebsiella pneumoniae</i>	29/60	48.33
2.	<i>E.coli</i>	27/37	72.97
3.	<i>Acinetobacter baumannii</i>	12/21	57.14
4.	<i>Proteus mirabilis</i>	3/10	30
5.	<i>Enterobacter spp.</i>	2/5	40
6.	<i>Morganella morganii</i>	1/3	33.33
Total	76/136	55.88	



**Figure 2: Frequency of sensitivity of isolates to elores**

**DISCUSSION**

MDR Gram negative bacteria are a cause of concern in management of infectious diseases. They not only increase mortality and morbidity in the patients but also increase the cost of treatment. They are being isolated at an increase rate from various clinical samples 10-12. The usage of carbapenems and colistin, our last bastion of antibiotics against MDR GNB, has significantly increased over past few years. Data published in Lancet Infect Dis in 2014 placed India as the leader in consumption of antibiotics followed by China and USA respectively. Same study also showed an increase in consumption of carbapenems and colistin to 45% and 13% over a period of 10 years from 2000 to 201013. Injudicious use of broad spectrum antibiotics, antibiotic therapy in inappropriate dose and duration, poor infection control practices and lack of antibiotic stewardship program in a hospital has contributed to the rapid emergence and spread of MDR organisms.

This study identified various MDR GNB isolates from clinical samples and examined the invitro activity of elores against these isolates. Our samples were representative of community and hospital isolates. Urine samples were both from the outpatient and catheterized inpatients, where as the semen samples were solely from the OPD. Rest all the samples ie blood, pus, bile, tracheal aspirate, bronchoalveolar lavage (BAL), drain fluid, tissue and pleural fluid were from inpatients only.

Urine contributed maximum number of isolates (50%) followed by pus (20.7%) and blood (14%). Enterobacteriaceae are the predominant isolates with non-fermenters accounting for the rest. The type of organism isolated is to a great extend function of the type of sample studied. Many of the other studies with similar sample composition have also report similar distribution 14-15.

*Klebsiella pneumoniae* (40%), *E.coli* (24.6%), *Acinetobacter baumannii* (14%) & *Paeruginosa* (8%) were the predominant MDR GNB organisms isolated. Other studies have also identified the same organisms as the most common MDR GNB isolated from clinical samples. Laxminarayan et al detected highest frequency of carbapenem resistance among *Acinetobacter baumannii* followed by *Klebsiella pneumoniae*, *P.aeruginosa* and *E.coli* isolates 4. In a study on MDR GNB from ICU of a tertiary care hospital in central India, out of 119 MDR GNB isolates, *Klebsiella pneumoniae* was commonest isolate (60) followed by *Paeruginosa* (26), *Acinetobacter spp.* (16), *E.coli* (12), *Proteus spp* (2), *Citrobacter spp.* (2) and *Burkholderia spp.* (1) respectively 16.

All the MDR GNB isolates except *Paeruginosa* and *Burkholderia cepacia* were tested against elores, the neoadjuvant antibiotic entity comprising of ceftriaxone and sulbactam with EDTA. 76 of the 136 (55.88%) MDR GNB isolates tested were found to be sensitive to elores. Maximum sensitivity was observed for *E.coli* (72.97%) and least for *Proteus mirabilis* (30%). The susceptibility of *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterobacter spp.* and *Morganella morganii* were 48.33%, 57.14%, 40% and 33.33% respectively. In a recent study by Arora et al, where they tested 200 isolates against elores, all the isolates of *Acinetobacter spp.* were uniformly susceptible, whereas sensitivity of *E. coli* and *Klebsiella pneumoniae* were 81% and 73% respectively 17. In a similar study by Sahu et, the susceptibility of ESBL and MBL producing isolates to ceftriaxone, sulbactam and EDTA combination varied from 64% to 100% and 42% to 89% respectively 18. Similar to our study, recently other studies have also shown higher susceptibility of Gram negative bacterial isolates to elores against 19-20.

Trials conducted on different patient groups to test clinical response to elores has shown promising results 21-23. Though the numbers are small, our study on the MDR GNB isolates have shown that a fair number of these isolates are susceptible to elores in vivo which is in concordance with previous studies. Elores appears to be a promising drug in our quest to treat MDR GNB infections and may be considered for management of such cases.

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