Research article

Antibiotic sensitivity pattern for non-beta lactam antibiotics and carbapenems in extended-spectrum beta-lactamase (ESBL) producing uropathogens versus non-ESBL producing uropathogens

WRPLI Wijesooriya[1], YB Herath[1], RAI Sugandhi[1],
A Weerawardhana[2], DS Ediriweera [3]

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Abstract

Introduction and Objectives: Urinary tract infections (UTIs) are frequent and predominantly caused by coliforms. ESBL producers are increasing in number limiting therapeutic options. It is therefore vital to institute precise, empiric antibiotic guidelines in order to prevent life-threatening urosepsis. The objective of this study was to compare antibiotic sensitivity (ABST) pattern of ESBL producers and non-ESBL producers against selected non-beta lactams and carbapenem antibiotics.

Methodology: Retrospective analysis of ABST of significant urinary coliform isolates was done.
Study setting: Department of Medical Microbiology, Faculty of Medicine, University of Kelaniya and Base Hospital, Wathupitiwala, Sri Lanka.
Study period: 01.01.2012 - 01.01.2016.
Study groups: ESBL producers and non-ESBL producers, 63 in each group. Sensitivity profiles of amikacin, gentamicin, netilmicin, nitrofurantoin, nalidixic acid, norfloxacin, ciprofloxacin, imipenem and meropenem were analyzed. Statistical analysis: R programming language. Level of significance $P<0.05$.

Results: ESBL producers were present in 63 patients, 36 (57.1%) of whom were females and 39 were inpatients (61.9%). Non-ESBL producers were isolated from urine of 63 patients, of whom 49 (77.8%) were females and 17 (26.9%) inpatients. Antibiotic sensitivity of ESBL producers ranged from 82.2% to 100% for netilmicin, amikacin, meropenem and imipenem, 65% for nitrofurantoin and from 14.8% to 32.1% for nalidixic acid, ciprofloxacin, norfloxacin and gentamicin. Antibiotic sensitivity of the non-ESBL producers ranged from 56.7% for nalidixic acid and from 76.8% to 85.1% for ciprofloxacin, nitrofurantoin, norfloxacin and gentamicin.

Conclusion: A female predominance was noted in both non ESBL and ESBL producers but there was a significant dominance of ESBL producers in male patients. ESBL producers were significantly common amongst inpatients than outpatients. ESBL-producers had significantly high resistance against nalidixic

[1]*Department of Medical Microbiology, Faculty of Medicine, University of Kelaniya, Sri Lanka.*
[2]*Base Hospital, Wathupitiwala, Sri Lanka.*
[3]*Computer Centre, Faculty of Medicine, University of Kelaniya, Sri Lanka.*

Address for correspondence: Dr Lakmini Wijesooriya, Department of Medical Microbiology, Faculty of Medicine, University of Kelaniya, Sri Lanka. +94718468865 Email: inokarajeev@gmail.com

https://orcid.org/0000-0001-6449-2321

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acid, ciprofloxacin, norfloxacin and gentamicin compared to non-ESBL producers. However, more than 2/3rd of isolates in both groups were sensitive to nitrofurantoin.

Keywords: UTI, ESBL, Non-beta lactam antibiotics

Introduction

Urinary tract infections (UTIs) are one of the commonest infections encountered in medical practice. Though it affects both genders, it is more common in females.[1,2] UTI occurs as either community or hospital acquired infection. Amongst several organisms which cause UTIs, *Escherichia coli* remains the most frequent in both community and hospital settings.[3] Treatment of UTI has become a challenge due to increasing antibiotic resistance over the past few years.[4,5]

Along with increasing antibiotic resistance, extended spectrum beta lactamase (ESBL) producers have been increasing.[6,7] Gram-negative organisms, in particular, members of the Enterobacteriaceae family, e.g. *E. coli* and *Klebsiella* species, produce β-lactamases with an extended spectrum of activity.[8,9,10] ESBL producers are resistant to the penicillins including the first, second and third-generation cephalosporins and aztreonam but not to the cephamycins or carbapenems. Even though such organisms are known to occur in nosocomial settings, they have become a threat to the community as well. The increasing prevalence of ESBL strains has resulted in prolonged hospital stay, limited therapeutic options and treatment failures resulting in an increased mortality.[11]

In order to prevent UTI worsening into life-threatening urosepsis, it is essential to commence more precise, empiric antibiotic since the result of antibiotic sensitivity testing (ABST) takes a minimum of three days following sample collection. This is important particularly in immunocompromised individuals, elderly, diabetic and critically ill patients.

The ABST pattern of uropathogens varies according to geographical distribution.[12,13,14] It is therefore important to determine the antibiotic sensitivity pattern in any particular community in order to select the best empiric antibiotic. It is also necessary to determine the sensitivity patterns of non-beta lactam antibiotics against ESBL and non-ESBL producers in order to prescribe appropriate empiric antibiotic/s.

The objective of the present study was to compare the ABS pattern of ESBL and non-ESBL producing uropathogenic coliform species against commonly used non-beta lactam antibiotics and carbapenems to enable optimum empiric antibiotic therapy.

Materials and methods

A retrospective analysis of ABST (Stoke's comparative disc diffusion method[15]) of significant ($\geq 10^5$ CFU/ml) urinary coliforms was performed using reports of urine cultures done at the Department of Medical Microbiology, Faculty of Medicine, University of Kelaniya, Sri Lanka and Base Hospital, Wathupitiwala, Sri Lanka from January 2012 to July 2016. Nitrofurantoin, nalidixic acid, ciprofloxacin, norfloxacin, and gentamicin were considered as non-beta lactam first line antibiotics whereas netilmicin, amikacin, meropenem and imipenem were considered as second line antibiotics.

Each group consisted of 63 isolates with no repeats. Isolates were divided as ESBL-producers and non-ESBL producers. Organism identification and ESBL production has been performed according to standard laboratory procedures.

ESBL production was determined phenotypically using double disk diffusion method.[16] A bacterial suspension of 0.5 McFarland turbidity standard was inoculated on a Muller-Hinton agar plate to obtain a confluent growth. Amoxicillin-clavulanic acid disc (20/10 µg) and a third generation cephalosporin (cefotaxime / ceftriaxone / ceftazidime) disc were placed 20 mm apart (center to center). The presence of ESBL was detected when distortion or enhanced inhibitory zone occurred towards the amoxicillin-clavulanic acid (keyhole effect).

Group comparisons were done using Pearson's chi-square test and log linear models as appropriate. Logistic regression models were used to evaluate differences in antibiotic sensitivity among ESBL producers and non-ESBL producers. Statistical significance was considered when $P<0.05$. Statistical analysis was performed using R programming language.

Results

During the study period, 63 ESBL producers and 63 non-ESBL producers were analyzed.
In the total study group of 126 patients, there were 85 (67.4 %) females and 41 (32.5%) males. The age distribution of the study group was 1 to 94 years.

Table 1: Comparison of ESBL producers (N=63) and non-ESBL producers (N=63)

		ESBL producers		Non-ESBL producers		
		N	%	N	%	P value
Age	< 20 years	5	29.1	12	70.6	0.01
	21- 40 years	25	61.0	16	39.0	0.16
	41- 60 years	13	41.9	18	58.0	0.37
	> 60 years	20	54.1	17	45.9	0.62
Gender	female	36	57.1	49	77.8	0.02
Inpatient		39	61.9	17	26.9	<0.01

Table 2: Antibiotic sensitivity of ESBL producers with non-ESBL producers for the first line antibiotics

	ABST of						
	ESBL producers			Non-ESBL producers			
Antibiotic	N tested	N sensitive	%	N tested	N sensitive	%	P value
Nitrofurantoin	60	39	65	62	51	82.3	0.0499
Nalidixic acid	61	9	14.8	60	34	56.7	<0.01
Ciprofloxacin	44	9	20.5	56	43	76.8	<0.01
Norfloxacin	57	12	21.1	57	46	80.7	<0.01
Gentamicin	56	18	32.1	40	47	85.1	<0.01

Table 3: Antibiotic sensitivity of ESBL producers for second line antibiotics

Antibiotic	N tested	N sensitive	%
Netilmicin	45	37	82.2
Amikacin	42	38	90. 5
Meropenem	46	44	95.7
Imipenem	34	34	100

Table 4: Comparison of antibiotic sensitivity between antibiotics against ESBL producers

Compared antibiotics	P value	Compared antibiotics	P value
nitrofurantoin vs ciprofloxacin	**0.006**	nalidixic acid vs ciprofloxacin	0.522
nitrofurantoin vs gentamicin	0.038	nalidixic acid vs gentamicin	0.082
nitrofurantoin vs nalidixic acid	**0.0003**	nalidixic acid vs norfloxacin	0.457
nitrofurantoin vs norfloxacin	**0.003**	netilmicin vs amikacin	0.762
ciprofloxacin vs gentamicin	0.320	netilmicin vs imipenem	0.552
ciprofloxacin vs norfloxacin	0.953	netilmicin vs meropenem	0.621
norfloxacin vs gentamicin	0.310	amikacin vs imipenem	0.762
norfloxacin vs gentamicin	0.856	meropenem vs amikacin	0.856
		meropenem vs imipenem	0.890

Table 5: Antibiotic sensitivity of non-EBSL producers: comparison between first line antibiotics

Compared antibiotics	P value	Compared antibiotics	P value
nitrofurantoin vs ciprofloxacin	0.804	nalidixic acid vs gentamicin	0.181
nitrofurantoin vs gentamicin	0.905	nalidixic acid vs norfloxacin	0.226
nitrofurantoin vs nalidixic acid	0.193	ciprofloxacin vs gentamicin	0.728
nitrofurantoin vs norfloxacin	0.944	ciprofloxacin vs norfloxacin	0.861
nalidixic acid vs ciprofloxacin	0.304	norfloxacin vs gentamicin	0.856

Discussion

In the background of emergence of ESBL producing uropathogens, it is vital to understand the suitability of non-beta lactam antibiotics as an empiric therapeutic option. The present study compared the ABS of ESBL and non-ESBL producers against non-beta lactam antibiotics.

ESBL producing coliforms were significantly lower than non-ESBL producers in the age group of < 20 years (P=0.01) compared with older patients (Table 1). This may be due to lesser exposure to antibiotics in the younger age group. However, there was no significant difference between ESBL and non-ESBL producers in patients age >20 years.

It is evident that UTIs are commoner in females, which is widely reported. In the present study, female predominance was seen in patients with ESBL producing pathogens (57.1%) as well as non-ESBL producing pathogens (77.8%) (Table 1). This finding is also similar to studies done locally and in other countries.[17,18] However, in the present study there was a significant dominance of ESBL producers in male patients compared to non-ESBL producers. This finding compares with that done by Heijer et al in which the antibiotic susceptibility of *E. coli* was lower in males in comparison with females, although the difference was not significant statistically.[19]

ESBL producers were significantly more common amongst inpatients (61.9%) compared to outpatients (38.1%) in the current study (Table 1). In contrast, non-ESBL producers were significantly higher in outpatients (73%) compared to inpatients (26.98) as shown in Table 1. The higher incidence of ESBL producers in inpatients is as expected due to high exposure to antibiotics in the hospital setting as reported from previous studies. [19, 20] However, it is important to note that about 1/3rd of ESBL producers were from the outpatient setting (Table 1). This is a challenge for planning of empiric antibiotic therapy today. According to a study conducted in 2012 in a tertiary care hospital of Sri Lanka, 13% of all community-acquired UTIs were due to ESBL producers.[21]

The antibiotic sensitivity of the ESBL producers in the present study demonstrated relatively higher resistance rates against commonly used empirical antibiotics [ciprofloxacin (20.5%), norfloxacin (21.1%) and nalidixic acid (14.8%)] in the primary care setting. These findings suggest that empiric therapy with commonly used antibiotics is questionable in a background with an increased ESBL prevalence. However, 65% ESBL producers were sensitive to nitrofurantoin for which the ESBL producers had the highest sensitivity among antibiotics used empirically for outpatients (Table 2).

Of the commonly used intravenous drugs in hospital setting, ESBL producers exhibit relatively poor sensitivity against gentamicin (32.1%) compared to other aminoglycosides, [amikacin (90.5%) and netilmicin (82.2%) (Table 2 & 3)]. Of the carbapenems, sensitivity for imipenem was 100% while it was 95.7% for meropenem (Table 3). This might be due to more use of meropenem compared to imipenem in therapy *(personal observation).* Similar patterns of ABS has been detected in a previously conducted local study.[22] This difference is not statistically significant, but needs further study with a larger sample size and evaluation of MIC values.

When comparing sensitivities of ESBL producers to different antibiotics, it is important to note that though nitrofurantoin is an oral antibiotic used in primary care, sensitivity for it was second only to second line antibiotics tested. Most importantly, sensitivity of ESBL producers to nitrofurantoin was significantly higher compared to the quinolones (ciprofloxacin, norfloxacin, nalidixic acid) which are commonly used for outpatients treatment of UTI ($P < 0.05$) (Table 4). Hence, the use of quinolones as empiric treatment may result in treatment failure leading to life threatening urosepsis.

With regard to ESBL producers' sensitivity to second line antibiotics, there was a relatively high percentage (>80%) of ESBL producers sensitive to netilmicin, amikacin, meropenem and imipenem (Table 3). No significant difference was observed in sensitivity among these four antibiotics ($P > 0.05$) (Table 4).

Of non-ESBL producers, only 56.7% was sensitive to nalidixic acid but satisfactory sensitivity of 75% was noted to ciprofloxacin (76.8%), norfloxacin (80.7%) and nitrofurantoin (82.3%) (Table 2) which have been identified as empiric antibiotics for treatment of UTI in studies conducted locally and globally.[8,20] Non-ESBL producers showed a sensitivity of 85.1% to gentamicin which is therefore a reasonable option for intravenous therapy. Within the group of non-ESBL producers, only about half of the isolates were sensitive to nalidixic acid while more than $3/4^{th}$ of isolates were sensitive to other tested antibiotics (nitrofurantoin, nalidixic acid, ciprofloxacin, norfloxacin and gentamicin). No significant difference was observed between sensitivity of non-ESBL producers to antibiotics tested as shown in Table 5.

ESBL-producers had significantly higher resistance than non-ESBL producers against nitrofurantoin, nalidixic acid, ciprofloxacin, norfloxacin and gentamicin ($p<0.05$) (Table 2). Though there is a significant difference in antibiotic sensitivity between these two groups, both groups had sensitivity greater than 65% for nitrofurantoin making it a preferred empiric antibiotic which could be used in the primary healthcare setting. Nalidixic acid was the most ineffective antibiotic for both ESBL producers and non-ESBL producers.

This study had some important limitations. Firstly, since it was a retrospective study, ABST data of non-ESBL producers to the second line antibiotics were not available, as they are not tested routinely when clinical isolates are sensitive to the majority of first line antibiotics. Therefore, sensitivity data for netilmicin, amikacin, meropenem and imipenem were not reported here for non-ESBL producers. Secondly, confirmatory tests for ESBL production and ascertainment of MICs were not performed. Clinical details of patients are not included due to insufficient information.

Conclusion

Non ESBL producers and ESBL producers were predominantly isolated from females with UTI. However, a significant dominance of ESBL producers was seen in male patients.

ESBL producing coliforms were significantly lower than non-ESBL producers in the age group of less than 20 years. There was a significantly higher number of ESBL producers amongst inpatients compared to outpatients.

ESBL-producers had significantly high resistance against nalidixic acid, ciprofloxacin, norfloxacin and gentamicin compared to non-ESBL producers. More than 2/3rd of isolates in both groups were sensitive to nitrofurantoin, indicating that this would be an optimal empiric antibiotic to be prescribed for UTI in an outpatient.

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