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Antimicrobial resistance patterns in extended-spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* isolates in a private tertiary hospital, Kenya

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Abstract

**Background:** Extended-Spectrum β-Lactamase (ESBL) producing Enterobacteriaceae have become widespread in hospitals and are increasing in community settings where they cause a variety of infections. In addition to hydrolyzing most β-lactam agents, bacteria harboring these enzymes display resistance to other unrelated antimicrobial agents and thus often pose a therapeutic dilemma. Though these resistance patterns have been studied elsewhere within Africa, such data from hospitals in Kenya are scanty. We therefore undertook to determine these patterns at the Aga Khan University hospital by studying multidrug resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates from patients’ samples.

**Findings:** A total of 159 isolates (109 *E. coli* and 50 *K. pneumoniae*) were confirmed as ESBL producers and tested against eighteen antimicrobials. The proportion of resistant isolates was high (>80%) for the antimicrobials tested with the exception of the carbapenems (<1%), nitrofurantoin (23%) and gentamicin (63%). *Klebsiella pneumoniae* had a higher proportion of isolates resistant to cefazidime, gentamicin and nitrofurantoin (P < 0.05) than *E. coli* which had a higher proportion of isolates resistant to ciprofloxacin, levofloxacin and tetracycline (P < 0.05).

**Conclusions:** In our study, antimicrobial coreistance is common in ESBL producers; however resistance to carbapenems is low and these drugs would be the appropriate empiric therapy for serious or life threatening systemic infections. Nitrofurantoin retains good activity among the multidrug resistant isolates and can be the drug of choice for non-complicated urinary tract infections due to ESBL producing *E. coli*.

**Keywords:** Extended-spectrum beta lactamases, *Escherichia coli*, *Klebsiella pneumoniae*, antimicrobial susceptibility, empiric therapy

**Background**

The precise definition of the term Extended-Spectrum β-Lactamase (ESBL) remains unclear but is generally used to refer to any β-lactamase, ‘generally acquired rather than inherent to a species, that is either able to confer resistance to oximino-cephalosporins (but not carbapenems), or that has an increased ability to do so, as compared with classic members of its genetic family’ [1].

ESBL producing strains of *Enterobacteriaceae* have emerged as a major problem in hospitalised as well as community based patients [2]. These organisms are responsible for a variety of infections like urinary tract infection (UTI), sepsis, hospital acquired pneumonia, intra-abdominal abscess, brain abscess and device related infections.

Organisms producing ESBLs are clinically relevant and remain an important cause of failure of therapy with cephalosporins [3]. ESBLs are primarily produced by the Enterobacteriaceae family, in particular *Klebsiella pneumoniae* and *Escherichia coli* [4].

Bacteria harboring ESBLs may also acquire and most often exhibit additional resistances to other antimicrobial classes such as the quinolones, tetracyclines, cotrimoxazole, trimethoprim, and aminoglycosides, which further limits therapeutic options and thus pose a therapeutic dilemma [5-8].

The Aga Khan University hospital (AKUH) is a private tertiary institution located in Nairobi, Kenya, with twenty satellite clinics spread across the country that send specimens for culture to the main laboratory. Among the Enterobacteriaceae, *E. coli* and *K. pneumoniae* are the two most isolated organisms from patients’ samples in the hospital’s microbiology section and ESBL producing isolates account for approximately 8% of the two species. Cephalosporins are among the most prescribed antibiotics in the hospital. In the year 2009 about 250,000 doses of ceftriaxone were consumed in the outpatient department alone. The antibiotic susceptibility profiles of these ESBL producing isolates have not been previously described in this hospital and are the focus of this study.

**Methods**

Three hundred thirty six multidrug resistant isolates cultured from clinical specimens and which were reported as *E. coli* and *K. pneumoniae* by use of routine bench biochemical tests and stored at -80°C in a broth medium with glycerol during the period 2007 to 2009 were included in the study. Only one isolate per patient was included. The isolates were revived on
Table 1. Distribution of clinical specimens and isolates cultured.

| Specimen type       | Inpatient | Outpatient |
|---------------------|-----------|------------|
|                     | E. coli   | K. pneumoniae | E. coli | K. pneumoniae | Total |
| ABDOMINAL DRAIN     | 1         | 0           | 0       | 0             | 1     |
| ASTIC FLUID         | 1         | 0           | 0       | 0             | 1     |
| AXILLA SWAB         | 2         | 0           | 0       | 0             | 2     |
| BRONCHIAL LAVAGE FLUID | 0     | 1           | 0       | 0             | 1     |
| BLOOD               | 1         | 1           | 0       | 0             | 2     |
| CATHETER TIP        | 0         | 1           | 0       | 0             | 1     |
| CSF                 | 1         | 0           | 0       | 0             | 1     |
| ENDOCEVICAL SWAB    | 0         | 0           | 1       | 0             | 1     |
| GROIN SWAB          | 1         | 0           | 0       | 0             | 1     |
| JOINT ASPIRATE      | 0         | 1           | 0       | 0             | 1     |
| NASAL SWAB          | 0         | 1           | 0       | 0             | 1     |
| PUS SWAB            | 5         | 8           | 6       | 2             | 21    |
| SPUTUM              | 2         | 3           | 0       | 1             | 6     |
| TRACHEAL ASPIRATE   | 1         | 3           | 0       | 0             | 4     |
| URINE               | 23        | 15          | 64      | 13            | 115   |
| **Total**           | **38**    | **34**      | **71**  | **16**        | **159** |

Table 2. Antibiotic susceptibilities of the isolates n=159.

| Antibiotic            | Resistant No (%) | Sensitive No (%) |
|-----------------------|------------------|------------------|
| Amoxicillin/Clavulanate | 159 (100%)        | 0                |
| Ampicillin            | 159 (100%)        | 0                |
| Ceftazidime           | 144 (90.6%)       | 15 (9.4%)        |
| Cefuroxime            | 158 (99.4%)       | 1 (0.6%)         |
| Cefoperazone          | 132 (83%)         | 27 (17%)         |
| Cefotaxime            | 158 (99.4%)       | 1 (0.6%)         |
| Gentamicin            | 101 (63.6%)       | 58 (36.4%)       |
| Imipenem              | 1 (0.6%)          | 158 (99.4%)      |
| Levofloxacin          | 128 (80.5%)       | 31 (19.5%)       |
| Meropenem             | 1 (0.6%)          | 158 (99.4%)      |
| Nitrofurantoin        | 37 (23.3%)        | 122 (76.7%)      |
| Piperacillin          | 159 (100%)        | 0                |
| Roxithromycin         | 159 (100%)        | 0                |
| Sulfamethoxazole/Trimethoprim | 144 (90.6%) | 15 (9.4%)     |
| Tetracycline          | 131 (82.4%)       | 28 (17.6%)       |
| Tobramycin            | 131 (82.4%)       | 28 (17.6%)       |
| Tazobactam/Piperacillin | 154 (96.9%)     | 5 (3.1%)         |

MacConkey media plates. Confirmation of ESBL production was done using the combined disc method (ceftazidime (CAZ 30μg) and cefotaxime (CTX 30μg) in combination with clavulanate (CLA 10μg), Becton Dickinson, New Jersey, USA) as per the Clinical Laboratory Standards Institute (CLSI 2009) guidelines on Mueller Hinton with 5% sheep blood agar plates, and antibiotic susceptibility performed using VITEK 2 (version 4.01, bioMerieux, Marcy-l’Etoile, France) and interpreted using the same CLSI 2009 guidelines. *Klebsiella pneumoniae* K6 ATCC 700603 (ESBL producer) and *Escherichia coli* ATCC 25922 (non-ESBL producer) served as the positive and negative controls respectively.

Eighteen antimicrobial agents were included; amoxicillin/clavulanate, ampicillin, ceftazidime, cefuroxime, ciprofloxacin, cefotaxime, cefotaxime, gentamicin, imipenem, levofloxacin, meropenem, nitrofurantoin, piperacillin, roxithromycin, sulfamethoxazole/trimethoprim, tetracycline, tobramycin, and tazobactam/piperacillin.

Data were analyzed using the statistical package for social sciences (SPSS 15.0, IBM SPSS, New York, USA).

Contingency table analysis was done by a chi-square test or two-tailed Fisher’s exact test where applicable. A p-value of less than 0.05 was considered as statistically significant. Ethical approval was granted by the Aga Khan University Hospital Nairobi Ethics Committee.

### Findings

#### Isolate characteristics

Out of the 336 isolates initially included, 177 isolates could not either be revived, did not have patient details, were neither *E. coli* nor *K. pneumoniae* on retesting with VITEK 2, or were not ESBL producers and therefore excluded leaving 159 isolates.

A hundred and nine of the isolates (68.6%) were *E. coli* and 50 (31.4%) were *K. pneumoniae*. Outpatient specimens were 87 (45.3%) and inpatient 72 (45.3%). *Escherichia coli* were isolated more frequently in outpatients than *K. pneumoniae* (p < 0.05).

Urine was the most common specimen processed with 115 (72%) of the samples. More than half of the urine samples were from outpatients. Other specimens were fewer and mostly from in-patients (Table 1). The ages of patients from whom the specimens were recovered ranged from 2 days to 90 years with a median of 42 years.

#### Antibiotic susceptibility patterns

A large proportion of the isolates (> 80%) were resistant to all classes of antibiotics tested apart from the carbapenems to which resistance was found in a single isolate. The proportion of isolates resistant to nitrofurantoin was substantially lower at 23% and 63% for gentamicin (Table 2).

There were significant species specific differences in the resistance patterns to some antibiotics. *Klebsiella pneumoniae* had a higher proportion of isolates resistant to ceftazidime, gentamicin and nitrofurantoin than *E. coli* isolates. In turn *E. coli* had a higher proportion of isolates resistant to ciprofloxacin, levofloxacin and tetracycline (Table 3).

### Discussion

Our study analyzed 159 isolates from in-patients and out-patients, thus encompassing both hospital and community acquired infections due to ESBL producing *E. coli* and *K. pneumoniae*.

The two bacteria species studied had high proportions of resistant isolates to most antimicrobial agents with the exception of carbapenems and nitrofurantoin.

Similar findings have been reported in other studies in Iran and Tanzania [9,10]. In these two studies imipenem was shown to be the most active antibiotic against the ESBL producing isolates and nitrofurantoin showed moderate activity.
The finding that nitrofurantoin remains active in the majority of isolates is clinically important to the outpatient setting, where most of the isolates from urine tended to be E. coli or K. pneumoniae.

Nitrofurantoin can therefore be used effectively for most non-life-threatening urinary tract infections with little regard to the antibiotic resistance mechanisms at play among ESBL producing isolates. From our experience many clinicians prefer to prescribe other drugs yet nitrofurantoin remains effective in most cases where there are multiple resistances as is also evident in other studies [11,12].

Given the popularity of fluoroquinolones in treating a variety of infections including respiratory, gastrointestinal and urinary tract infections, the two drugs tested (ciprofloxacin and levofloxacin) fared poorly with >80% of the isolates showing resistance to both. This is not surprising among E. coli and K. pneumoniae displaying multiple resistances to various classes of antimicrobials and are therefore important to identify and treat accordingly. In our study the carbapenems and nitrofurantoin retain good activity and would thus be the drugs of choice for empiric therapy in life threatening infections and non-complicated urinary tract infections respectively. There is need to carry out more studies to determine the epidemiology of ESBL related infections and the associated clinical burden locally.

Table 3. Significant species specific differences in antimicrobial resistance.

| Antibiotic      | Sensitivity | E. coli (n=109) | K. pneumoniae (n=50) | P-value |
|-----------------|-------------|-----------------|----------------------|---------|
| Cefazidine      | R           | 95              | 49                   | 0.038   |
|                 | S           | 14              | 1                    |         |
| Ciprofloxacin   | R           | 101             | 31                   | <0.001  |
|                 | S           | 8               | 19                   |         |
| Levofloxacin    | R           | 99              | 29                   | <0.001  |
|                 | S           | 10              | 21                   |         |
| Nitrofurantoin  | R           | 12              | 25                   | <0.001  |
|                 | S           | 97              | 25                   |         |
| Tetracycline    | R           | 96              | 35                   | 0.005   |
|                 | S           | 13              | 15                   |         |

R= resistant, S= sensitive

cephaplosporins with activity against ESBL producers [16]. Our data suggests that cephalosporins would be of limited use in the treatment of ESBL related infections in our institution as the proportion of resistant isolates was high. These observations also apply to the β-lactam/β-lactam inhibitor combinations for which we observed a high proportion of isolates with MICs in the resistant range. A possible explanation for the latter observation is the presence of additional resistance mechanisms which as noted above may be acquired together with ESBLs. However, these additional mechanisms were not specifically looked for in this study.

The screening for ESBL production is not routinely done in many clinical laboratories in this country and the impact of treatment failure associated with these infections when treated with cephalosporins is not well documented.

Conclusions

ESBL producing E. coli and K. pneumoniae display multiple resistances to various classes of antimicrobials and are therefore important to identify and treat accordingly. In our study the carbapenems and nitrofurantoin retain good activity and would thus be the drugs of choice for empiric therapy in life threatening infections and non-complicated urinary tract infections respectively. There is need to carry out more studies to determine the epidemiology of ESBL related infections and the associated clinical burden locally.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

DM worked on the selection of isolates, data analysis and drafted the manuscript. PM was involved in study design and data analysis. AN provided technical input on the bench and reviewed the susceptibilities data. GR conceived of the study, gained ethical approval and coordinated the study. All the authors read and approved the final manuscript.

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References

1. Livermore DM: Defining an extended-spectrum beta-lactamase. Clin Microbiol Infect 2008, 14 Suppl 1:3-10. | Article | PubMed
2. Rodriguez-Bano J, Navarro MD, Romero L, Martinez-Martinez L, Muniaín MA, Perea EJ, Perez-Cano R and Pascual A: Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase-producing Escherichia coli in nonhospitalized patients. J Clin Microbiol 2004, 42:1089-94. | Article | PubMed Abstract | PubMed Full Text
3. Bradford PA: Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 2001, 14:933-51, table of contents. | Article | PubMed Abstract | PubMed Full Text
4. Paterson DL and Bonomo RA: Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 2005, 18:657-86. | Article | PubMed | PubMed Abstract | PubMed Full Text
5. Chopra I, Schofield C, Everett M, O’Neill A, Miller K, Wilcox M, Frere JM, Dawson M, Craplewski L, Urleb U and Courvalin P: Treatment of health-care-associated infections caused by Gram-negative bacteria: a consensus statement. *Lancet Infect Dis* 2008, 8:133-9. | Article | PubMed

6. Morosini MI, Garcia-Castillo M, Coque TM, Valverde A, Novais A, Loza E, Baquero F and Canton R: Antibiotic co-resistance in extended-spectrum-beta-lactamase-producing Enterobacteriaceae and in vitro activity of tigecycline. *Antimicrob Agents Chemother* 2006, 50:2695-9. | Article | PubMed Abstract | PubMed Full Text

7. Pitout JD and Laupland KB: Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect Dis* 2008, 8:159-66. | Article | PubMed

8. Talbot GH: What is in the pipeline for Gram-negative pathogens? *Expert Rev Ant Infect Ther* 2008, 6:39-49. | Article | PubMed

9. Mehrgan H, Rahbar M and Arab-Halvaii Z: High prevalence of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a tertiary care hospital in Tehran, Iran. *J Infect Dev Ctries* 2010, 4:132-8. | Article | PubMed

10. Moyo SJ, Aboud S, Kasubi M, Lyamuya EF and Maselle SY: Antimicrobial resistance among producers and non-producers of extended spectrum beta-lactamases in urinary isolates at a tertiary Hospital in Tanzania. *BMC Res Notes* 2010, 3:348. | Article | PubMed Abstract | PubMed Full Text

11. Garau J: Other antimicrobials of interest in the era of extended-spectrum beta-lactamases: fosfomycin, nitrofurantoin and tigecycline. *Clin Microbiol Infect* 2008, 14 Suppl 1:198-202. | Article | PubMed

12. Schito GC, Naber KG, Botto H, Palou J, Mazzei T, Gualco L and Marchese A: The ARESC study; an international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. *Int J Antimicrob Agents* 2009, 34:407-13. | Article | PubMed

13. Paterson DL, Mulazimoglu L, Casellas JM, Ko WC, Goossens H, Von Gottberg A, Mohapatra S, Trenholme GM, Klugman KP, McCormack JG and Yu VL: Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum beta-lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. *Clin Infect Dis* 2000, 30:473-8. | Article | PubMed

14. Maina D, Revathi G, Kariuki S and Ozwara H: Genotypes and cephalosporin susceptibility in extended-spectrum beta-lactamase producing enterobacteriaceae in the community. *J Infect Dev Ctries* 2012, 6:470-7. | Article | PubMed

15. Ben-Ami R, Schwaber MJ, Navon-Venezia S, Schwartz D, Giladi M, Chmelinsky I, Leavitt A and Carmeli Y: Influx of extended-spectrum beta-lactamase-producing enterobacteriaceae into the hospital. *Clin Infect Dis* 2006, 42:925-34. | Article | PubMed

16. Ramphal R and Ambrose PG: Extended-spectrum beta-lactamases and clinical outcomes: current data. *Clin Infect Dis* 2006, 42 Suppl 4:S164-72. | Article | PubMed

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