Prevalence and antimicrobial susceptibility of extended-spectrum $\beta$-lactamase-producing \textit{Escherichia coli} and \textit{Klebsiella pneumoniae} in a general hospital

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\textbf{BACKGROUND:} The prevalence of extended-spectrum $\beta$-lactamases (ESBLs) varies between countries and institutions. We studied the prevalence of ESBL among clinical isolates of \textit{Escherichia coli} and \textit{Klebsiella pneumoniae} and analyzed patterns of susceptibilities to different antimicrobial agents in a general hospital in Saudi Arabia over a 15-month period.

\textbf{METHODS:} A total of 2455 clinical isolates of \textit{E. coli} and \textit{K. pneumoniae} were tested for ESBL production by double-disk diffusion. The minimum inhibitory concentration to imipenem, meropenem, piperacillin-tazobactam, cefepime, ciprofloxacin, gentamicin and amikacin were determined by the agar dilution method.

\textbf{RESULTS:} Of the 2455 isolates of \textit{E. coli} and \textit{K. pneumoniae} tested, 268 (11\%) produced ESBL. The ESBL phenotype was detected in 10.3\% of 1674 \textit{E. coli} isolates and 12.2\% of 781 \textit{K. pneumoniae} isolates. The majority of these isolates were from urine (57.5\%) and wounds (17\%). Only 7\% of the blood culture isolates were ESBL-producing. Overall, carbapenems (imipenem and meropenem) had good activity against the ESBL-producing isolates tested (over 92\% of isolates were susceptible). There was no difference in the activity of imipenem and meropenem against the ESBL-producing \textit{E. coli} or \textit{K. pneumoniae}. Over 66\% of the isolates were susceptible to piperacillin-tazobactam. Susceptibilities of the isolates to amikacin varied, ranging from 72.8\% for \textit{E. coli} to 62\% for \textit{K. pneumoniae}. Gentamicin, ciprofloxacin and cefepime were active against 58.6\%, 55\% and 22.8\% of the isolates, respectively.

\textbf{CONCLUSION:} Our findings demonstrate the increasing incidence of infection with ESBL-producing bacteria, and the high rates of antimicrobial resistance encountered among them. Clinicians should be familiar with the clinical importance of these enzymes and potential strategies for dealing with them.

Infections by extended-spectrum $\beta$-lactamase (ESBL)-producing organisms are a worldwide problem. There is a growing concern for the increasing antimicrobial resistance among the ESBL-producing \textit{Escherichia coli} and \textit{Klebsiella pneumoniae}.

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\textbf{Accepted for publication:}
March 2005

Ann Saudi Med. 2005;25(3):239–242
ESBL-producing organisms are often multidrug resistant, as the plasmids producing ESBLs can carry resistance to other antibiotics. The therapeutic choices in infections caused by ESBL-producing organisms are limited because of cross-resistance. The carbapenems are the most active antibiotics against these organisms. The increasing antimicrobial resistance among ESBL-producing bacteria may lead to more use of expensive broad-spectrum drugs such as carbapenems and a significantly longer duration of hospital stay. Additionally, these resistant isolates may not be detected with routine in vitro laboratory susceptibility testing, which can result in adverse therapeutic outcomes. Antimicrobial surveillance studies can be used to assess the changes in patterns of susceptibility of bacterial pathogens to antimicrobial agents. As antimicrobial susceptibility of ESBL-producing pathogens varies from one region to another, we conducted this study to determine ESBL production by the clinical isolates of E. coli and K. pneumoniae and to analyze the patterns of susceptibility of these isolates to different antimicrobial drugs.

Methods
This study was carried out at the Almana General Hospitals in Alkhobar and Dammam. The hospitals have 500 beds in total, including 28 critical care beds and beds in major speciality areas such as cardiothoracic and vascular surgery, neurosurgery, plastic surgery, urology and dialysis units, orthopedics, and obstetrics and gynecology. The hospitals have restrictive antibiotic policies for the broad-spectrum antibiotic agents, including third and fourth generations cephalosporins, carbapenems, glycopeptides and quinolones. The study period was from June 2003 to August 2004. A total of 2455 clinical isolates of Escherichia coli and Klebsiella pneumoniae were studied for ESBL production (one clinical isolate per patient). The strains were identified using standard techniques and the API 20E (BioMerieux, France). ESBL detection was done by the double-disc diffusion using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid. An increase in zone size of more than or equal to 5 mm for cefotaxime and ceftazidime with and without clavulanic acid was taken as an indication of ESBL production. The minimum inhibitory concentrations (MICs) of imipenem, meropenem, piperacillin-tazobactam, ceftazidime, ciprofloxacin, gentamicin and amikacin were evaluated against the ESBL-producing isolates. MIC values were determined with the agar dilution methods according to the National Committee for Clinical Laboratory Standards (NCCLS). Percentage susceptibility to the antimicrobial agents was determined at the NCCLS susceptibility concentrations of ≤4 mg/L for imipenem, meropenem and gentamicin; ≤1 mg/L for ciprofloxacin; ≤8 mg/L for ceftazidime; ≤16 mg/L for amikacin and piperacillin-tazobactam. Percentage resistance to the antimicrobial agents was determined at concentrations of ≥16 mg/L for imipenem, meropenem, ≥8 mg/L for gentamicin; ≥4 mg/L for ciprofloxacin; ≥32 mg/L for ceftazidime and amikacin; ≥128 mg/L for piperacillin-tazobactam. Quality control was performed by including E. coli ATCC 25922 as a negative control and K. pneumoniae (ATCC 700603) as a positive control.

Results
Of the 2455 isolates of E. coli and K. pneumoniae, 268 (11%) were confirmed as ESBL producers. Of the 1674 E. coli isolates, 173 (10.3%) were positive for ESBL. Among the 781 K. pneumoniae isolates tested, 95 (12.2%) were ESBL-producers. The 268 ESBL-producing isolates were recovered from the following: 154 in urine, 46 in wounds, 30 in bed sores, 19 in blood, 10 in sputum, and 9 others (Table 1). The susceptibility data of the ESBL-producing E. coli and K. pneumoniae are summarized in Table 2. More than 92% of these organisms were susceptible to carbapenems. Imipenem and meropenem were equally active against the ESBL-producing E. coli and K. pneumoniae. Amikacin was active against 69% of the isolates. The susceptibility of the ESBL-producing isolates to gentamicin, ciprofloxacin and ceftazidime was 58.6%, 55% and 22.8%, respectively. MICs are summarized in Table 3.

Discussion
Although many international studies have addressed the emergence of ESBL-producing K. pneumoniae and E. coli, there are few local reports on this issue. The current study demonstrated an increasing prevalence of ESBL-producing E. coli (from 6.5% in 2002 to 10.3% in 2004) and K. pneumoniae (from 5.4% in 2002 to 12.2% in 2004). The increase among K. pneumoniae was higher than that among E. coli. A recent study from Lebanon has shown a similar increasing trend in the percentage of ESBL-producing E. coli and K. pneumoniae.

Multidrug resistance has been reported among ESBL-producing organisms. Our results showed the isolates to be highly resistant to ceftazidime, ciprofloxacin, gentamicin and amikacin.
ciprofloxacin and gentamicin. There was a significant increase in the resistance of the ESBL-producing isolates to ciprofloxacin compared to those we reported previously (from 55% to 72%). On the whole, for all ESBL-producing isolates, MICs for gentamicin and ciprofloxacin were high (32–64 mg/L). The elevated MIC of ciprofloxacin in E. coli and K. pneumoniae indicates that in these species resistance to ciprofloxacin is strongly associated with ESBL production. Other studies have reported the increasing frequency of the association between ciprofloxacin resistance and ESBL production. This association greatly limits the role of ciprofloxacin against ESBL-producing bacterial pathogens.

Our data showed a difference in susceptibility pattern between ESBL-producing isolates of E. coli and K. pneumoniae for amikacin. K. pneumoniae isolates showed lower susceptibility to amikacin than E. coli. Other studies have shown variable susceptibility patterns compared to ours. A recent study from the USA showed lower susceptibility to amikacin in E. coli than K. pneumoniae. Another study from Taiwan reported higher susceptibility to amikacin in E. coli than K. pneumoniae.

Of all the antimicrobial agents tested, carbapenems (imipenem and meropenem) had the highest activity against the ESBL-producing organisms, similar to other studies. Amikacin and piperacillin-tazobactam showed the highest activity after carbapenems. The carbapenems are known to be stable against ESBL enzymes and effective in the treatment of infections caused by ESBL-producing bacteria.

In conclusion, this study shows an increasing frequency of ESBL among clinical isolates and a high rate of multidrug resistance, which may have been caused by the excessive use of broad-spectrum antibiotics. As the available treatment options are limited, prevention of ESBL infections by restricting the use of antimicrobial agents along with implementation of infection control measures remain of primary importance. Because of the new challenges presented by the changing nature and distribution of these enzymes, clinicians need to be familiar with the clinical significance of these enzymes, and clinical microbiology laboratories need to adopt a technique most suitable to them for their detection. Conducting molecular and epidemiological studies will help to identify the different types of ESBL and establish the relationship between ESBL-producing isolates.

Table 1. Source and distribution of extended-spectrum β-lactamase (ESBL) producing clinical specimens.

| Specimen          | Number (%) of E. coli isolates | Number (%) of K. pneumoniae isolates | Total number (%) of isolates |
|-------------------|--------------------------------|--------------------------------------|------------------------------|
| Urine             | 105 (60)                       | 49 (51.5)                            | 154 (57.5)                   |
| Wound             | 34 (19.6)                      | 12 (12.6)                            | 46 (17)                      |
| Bedsores          | 13 (7.5)                       | 17 (17.9)                            | 30 (11)                      |
| Blood             | 7 (4)                          | 12 (12.6)                            | 19 (7)                       |
| Sputum            | 7 (4)                          | 3 (3)                                | 10 (3.7)                     |
| Bronchial wash    | 2 (1)                          | 1 (1)                                | 3 (1)                        |
| Peritoneal fluid  | 3 (1.7)                        | 1 (1)                                | 4 (1.5)                      |
| High vaginal swab | 2 (1)                          | –                                    | 2 (0.7)                      |
| Total             | 173 (64.5)                     | 95 (35.5)                            | 268                          |

Table 2. Susceptibility patterns of ESBL-producing organisms.

| Antimicrobial agent | E. coli Mean MIC (mg/L) and % resistant (n=173) | K. pneumoniae Mean MIC (mg/L) and % resistant (n=95) |
|---------------------|-----------------------------------------------|-----------------------------------------------|
| Imipenem            | 16 (12)                                       | 2 (7)                                         |
| Meropenem           | 16 (12)                                       | 2 (7)                                         |
| Amikacin            | ≥32 (27.2)                                    | ≥32 (38)                                      |
| Piperacillin-tazobactam | ≥128 (53)                   | ≥128 (56.2)                                    |
| Cefepime            | ≥32 (73.9)                                    | ≥64 (83)                                      |
| Ciprofloxacin       | ≥32 (45.6)                                    | ≥64 (43)                                      |
| Gentamicin          | ≥64 (42)                                      | ≥64 (40)                                      |

* Resistance as defined by NCCLS 15
Abstract # 188Sa.
Diseases Society of America, Denver, Colorado.

Monomycin

In-vitro susceptibility and clinical outcome of bac-
tance and opportunities for its control.

Klebsiella pneumoniae
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