academicJournals

Vol. 9(41), pp. 2145-2158, 14 October, 2015
DOI: 10.5897/AJMR2015.7584
Article Number: 5572AEC55926
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

*Full Length Research Paper*

# Antimicrobial susceptibility pattern of Gram-negative bacilli isolated from a Teaching Hospital in Jeddah, Saudi Arabia

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Received 17 May, 2015; Accepted 25 September, 2015

**Gram-negative bacilli (GNB) are commonly implicated in clinical diseases. However, with their increasing resistance to antimicrobial agents, treatment becomes a challenge. This study has been conducted at King Abdulaziz University Hospital in Jeddah, Saudi Arabia over a period of one month from July to August 2011. Identification and antibiotic sensitivity tests of GNB were performed using standard microbiological methods and Vitek2 system. Extended spectrum beta lactamase (ESBLs) strains were detected using double disc synergy test and Vitek 2 system. A total of 176 Gram-negative bacilli were studied. The most frequently isolated organism was *E. coli* (38.07%) followed by *Klebsiella pneumoniae* (15.91%), *Pseudomonas aeruginosa* (11.93%), *Proteus mirabilis* (9.66%) and *Acinetobacter baumannii* (6.82%). Other Gram-negative bacilli were less frequent. Isolates were detected most frequently from ICU patients (26.70%). Urinary tract, wound and respiratory tract infections were implicated most often. Extended spectrum beta lactamase strains accounted for 20% of all Enterobacteriacae. The vast majority of the GNB isolates were resistant to many antibiotics. Carbapenems, tigecycline and amikacin were effective against most multi-drug resistant Enterobacteriacae. *Pseudomonas aeruginosa* was resistant to several antibiotics; most effective agents were ceftazidime (80.95%), aztreonam (76.19%), carbapenem (90.48%), amikacin (90.48%), fluoroquinolones (80.95%) and piperacillin (61.90%). Isolates of *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* were mainly multi-drug resistant to most tested antibiotics. In view of high levels of antibiotic resistance encountered, continuous surveillance of antimicrobial susceptibility patterns is warranted.**

**Key words:** Gram-negative bacilli, resistance, extended-spectrum beta lactamase, intensive care unit, antibiotics, Enterobacteriaceae, King Abdulaziz University Hospital, multi-drug resistant.

## INTRODUCTION

Gram-negative bacilli (GNB) are a large heterogenous group and amongst them the Enterobacteriacae are one of the most common bacteria isolated in clinical laboratories. Enterobacteria are ubiquitous in the environment and carried in the intestinal tract of human and many animals. Generally, they are not associated with disease. However, when clinical infections occur, *Escherichia coli* (*E. coli*) is most often implicated. Clinical

infections frequently involve the urinary and intestinal tract although other sites may be involved. Other members of the group such as *Serratia marcescens* and *Enterobacter aerogenes* (*Ent. aerogenes*) are opportunistic. Among the other GNB, *Ps. aeruginosa* is renowned for hospital acquired infections (Peleg and Hooper, 2010).

Antibiotics are invariably used for treatment of these infections. However, the tremendous therapeutic advantage offered by these agents is being negated by the emergence of increasingly resistant strains of bacteria on a global scale (Yezli et al., 2014). Also, in Saudi Arabia (SA), prevalence of antimicrobial resistance amongst a variety of clinical pathogens has been documented (Memish et al., 2012; Yezli et al., 2014). High levels of antibiotic resistance among GNB specially *Ps. aeruginosa* and *A. baumannii* has been reported particularly from intensive care units (ICUs) originating from a variety of infections and resulting in significant morbidity and mortality (Al-Ahmadey and Mohamed, 2013).

In recent years, the problem of drug resistance has been enhanced by the emergence of organisms producing extended spectrum beta-lactamases (ESBLs). These strains exhibit resistance to various antimicrobial agents including third generation cephalosporins, extended spectrum penicillins, and monobactams. Susceptibility to other agents such as fluoroquinilones and aminoglycosides is variable. The carbapenems are often the only active class of antibiotics against these strains (Kader and Kumar, 2004). Prevalence of these ESBLs varies globally and in SA; differing rates have been reported. Extended spectrum beta-lactamase strains have been implicated in both hospital and community settings and their increasing levels have been a cause for concern (Khanfar et al., 2009; Yezli et al., 2014). Hence, regular surveillance of clinical isolates and their susceptibility patterns is warranted.

The aim of this prospective study was to determine the antimicrobial susceptibility patterns of GNB and ESBL producing strains in clinical samples.

## MATERIALS AND METHODS

### Setting and design

This prospective study was undertaken in a tertiary care hospital in Jeddah; King Abdulaziz University Hospital (KAUH) with 845 bed capacity, during a period of one month from 15$^{th}$ of July to 15$^{th}$ of August 2011.

A total of 176 Gram-negative bacilli isolates were identified from 158 patients during the study period. Clinical Gram-negative bacilli strains were obtained from various patient specimens submitted to Clinical Microbiology Laboratory at KAUH.

### Data collection

Demographic data (Age, gender, nationality, specimen type and ward of hospital) of patients with Gram-negative bacterial infections (UTIs, skin infections, sepsis, pneumonia, etc.) were recorded on a standardized form.

### Bacterial isolates

Strains were isolated from a variety of specimens cultured on MacConkey agar and 5% Sheep Blood agar (Saudi Prepared Media Laboratory, SPML). All isolates used in the study were maintained on nutrient agar slants at 4ºC.

### Identification of Gram-negative bacilli

Preliminary identification of GNB was performed using conventional methods including: Gram-staining, culture characteristics, lactose fermentation, and oxidase test. Further identification to species level was performed using Vitek 2 (ID-GN card) automated system (BioMerieux, France) according to manufacturer's instructions.

### Antimicrobial susceptibility test

#### *Disc diffusion method*

The susceptibility of the tested isolates was carried out by Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standard Institutes (CLSI) guidelines (CLSI, 2012).

The commercial antibiotics discs (Oxoid Limited, UK) used for Gram-negative bacilli were ampicillin (10 µg), piperacillin (100 µg), piperacillin-tazobactam (110 µg), amoxicillin/clavulanic acid (30 µg), aztreonam (30 µg), cefepime (30 µg), cefotaxime (30 µg), cefuroxime (30 µg), cefoxitin (30 µg), cefpodoxime (10 µg), ceftazidime (30 µg), ceftriaxone (30 µg), meropenem (10µg), imipenem (10 µg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), nitrofurantoin (300 µg), tigecycline (15 µg), colistin (10 µg), and trimethoprim/ sulfamethoxazole (25 µg).

#### *Minimum inhibitory concentrations (MICs)*

Minimum inhibitory concentrations of various antibiotics to different isolates were determined using automated microbiology system Vitek 2. Gram-negative cards (AST-N114/AST-GN26/AST-EXN8) were used according to the instructions of the manufacture.

### Quality control strains

*E. coli* ATCC 25922, *K. pneumonia*e ATCC 700603 and *Ps. aeruginosa* ATCC 27853 were used as controls for the antimicrobial susceptibility tests.

### Screening for extended spectrum-beta-lactamase (ESBLs) by double-disc synergy test

Gram-negative bacilli (*E. coli* and *K. pneumonia*e) resistant to third

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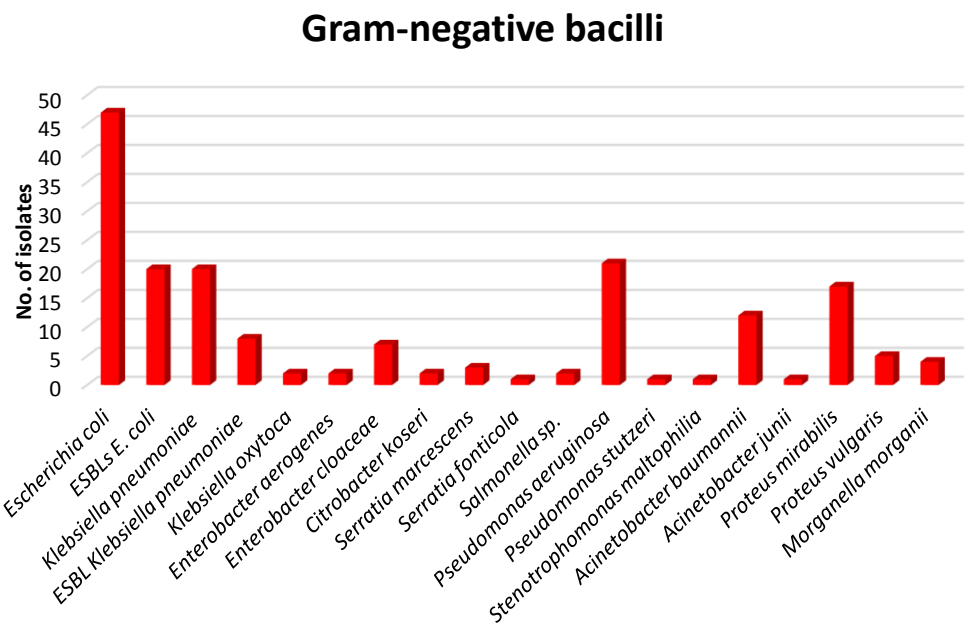


**Figure 1.** Distribution of most frequently isolated Gram-negative bacilli.

generation cephalosporins were tested for ESBL production by double disc synergy test (DDST). The DDST was performed as a standard disc diffusion method using Mueller-Hinton agar plates according to CLSI guidelines (CLSI, 2012). Discs containing 30 µg aztreonam and 30 µg of cefotaxime, ceftazidime, cefpodoxime, ceftriaxone, cefuroxime and cefepime each were placed 15-20 mm apart (center to center) around a disc containing amoxicillin+clavulanic acid (augmentin 20 µg+10 µg respectively); clavulanic acid is an inhibitor of beta-lactamase. The Mueller-Hinton agar plates were incubated at 37°C for 24 h. Enhancement of inhibition zone of any one of the test antibiotics towards augmentin disc was regarded as presumptive ESBL production, these isolates were subjected to Vitek 2 system (AST-EXN8) for confirmatory test.

#### Statistical analysis

The results were statistically analyzed using Microsoft Excel 2010. Statistical tests were presented as frequencies and percentages.

## RESULTS

During this prospective study, 176 Gram-negative bacilli isolates derived from 158 patients were collected. Males accounted for 41.1% (65/158) and females 58.9% (93/158). The proportion of non-Saudi patients 67.7% (107/158) was greater than Saudis 32.3% (51/107). The most common age group was above 50 years.

Amongst the GNB, *E. coli* (38.07%) (67/176) was detected most often followed by *K. pneumonia*e (15.91%) (28/ 176), *Ps. aeruginosa* (11.93%) (21/176), *P. mirabilis* (9.66%) (17/ 176) and *A. baumannii* (6.82%) (12/176). Other genera were detected infrequently as shown in Figure 1.

The distribution of non-ESBLs GNB and ESBLs Enterobacteriaceae with respect to clinical specimen type is shown in Tables 1 and 2.

Gram-negative bacilli were isolated most frequently from urine (43.75%) followed by wound and abscess specimens (27.84%), then respiratory secretions (19.89%) and lastly blood (6.25%). Similarly, *E. coli* (73.13%), *K. pneumoniae* (39.29%) and *A. baumannii* (41.67%) were also cultured most often from urine. In contrast, *Ps. aeruginosa* (52.38%) were frequently derived from respiratory secretions whilst *P. mirabilis* (82.35%) was mostly isolated from wound and abscess specimens.

Results of the distribution of non-ESBLs GNB and ESBLs Enterobacteriaceae according to ward type are shown in Tables 3 and 4.

Gram-negative bacilli were detected most frequently from ICU patients (26.7%). Similarly, *K. pneumoniae* (35.71%), *Ps. aeruginosa* (33.33%) and *A. baumannii* (58.33%) were also most often in ICUs patients. Although 25% (5/20) of ESBLs *E. coli* were detected in ICUs patients, in contrast, 38.30% (18/47) of non-ESBLs *E. coli* were isolated from outpatients (obstetrics/gynecology clinics), whilst the prevalence of *P. mirabilis* (41.18%) was greatest among patients attending medical units.

Of the 140 *Enterobacteriaceae* isolated, 20% (28/140) were ESBLs producing strains. Among these, 71.43% (20/28) were *E. coli* and 28.57% (8/28) were *K. pneumoniae*.

The test was considered positive upon the formation of a clear extension of the edges of the zones of inhibition of any of the cephalosporin antibiotics towards the disc containing clavulanic acid, often showing a characteristic

**Table 1.** Distribution of non-ESBLs Gram-negative bacilli with respect to clinical specimen.

| Bacterial isolates | Number of isolates | Specimen | | | | |
|---|---|---|---|---|---|---|
| | | Blood | Wound and abscess | Urine | Respiratory secretion | Others*** |
| *Escherichia coli* | 47 | 1(2.13%)* (11.11%)** | 8 (17.02%)* (18.60%)** | 37 (78.72%)* (61.67%)** | 1 (2.13%)* (3.13%)** | ND |
| *Klebsiella pneumoniae* | 20 | 1 (5%)* (11.11%)** | 5 (25%)* (11.63%)** | 6 (30%)* (10%)** | 7 (35%)* (21.88%)** | 1 (5%)* (25%)** |
| *Klebsiella oxytoca* | 2 | ND | ND | 1 (50%)* (1.67%)** | 1 (50%)* (3.13%)** | ND |
| *Enterobacter aerogenes* | 2 | ND | 2 (100%)* (4.65%)** | ND | ND | ND |
| *Enterobacter cloaceae* | 7 | 1 (14.29%)* (11.11%)** | 2 (28.57%)* (4.65%)** | 1 (14.29%)* (1.67%)** | 1 (14.29%)* (3.13%)** | 2 (28.57%)* (50%)** |
| *Citrobacter koseri* | 2 | ND | ND | 1 (50%)* (1.67%)** | 1 (50%)* (3.13%)** | ND |
| *Serratia marcescens* | 3 | ND | ND | ND | 2 (66.67%)* (6.25%)** | 1 (33.33%)* (25%)** |
| *Serratia fonticola* | 1 | ND | ND | 1 (100%)* (1.67%)** | ND | ND |
| *Salmonella* sp. | 2 | 2 (100%)* (22.22%)** | ND | ND | ND | ND |

**Table 1.** Contd.

| | | | | | | |
|---|---|---|---|---|---|---|
| *Pseudomonas aeruginosa* | 21 | ND | 6 (28.57%)* (13.95%)** | 4 (19.05%)* (6.67%)** | 11 (52.38%)* (34.38%)** | ND |
| *Pseudomonas stutzeri* | 1 | ND | ND | 1 (100%)* (1.67%)** | ND | ND |
| *Stenotrophomonas maltophilia* | 1 | ND | ND | ND | 1 (100%)* (3.13%)** | ND |
| *Acinetobacter baumannii* | 12 | 2 (16.67%)* (22.22%)** | 1 (8.33%)* (2.32%)** | 5 (41.67%)* (8.33%)** | 4 (33.33%)* (12.5%)** | ND |
| *Acinetobacter junii* | 1 | 1 (100%)* (11.11%)** | ND | ND | ND | ND |
| *Proteus mirabilis* | 17 | ND | 14 (82.35%)* (32.56%)** | 2 (11.76%)* (3.33%)** | 1 (5.88%)* (3.13%)** | ND |
| *Proteus vulgaris* | 5 | ND | 3 (60%)* (6.98%)** | ND | 2 (40%)* (6.25%)** | ND |
| *Morganella morganii* | 4 | 1 (25%)* (11.11%)** | 2 (50%)* (4.65%)** | 1 (25%)* (1.67%)** | ND | ND |
| Total | 148 | 9 | 43 | 60 | 32 | 4 |

*% according to total number of the specified isolate; **% according to specimen type; ***Other specimens such as eye swab and body fluids. ND, Not detected.

shape-zone referred to as ‘keyhole’ (Figures 2 and 3) due to inhibition of β-lactamase by clavulanic acid (Neu and Fu, 1978).

The antibiotic resistance patterns of non-ESBLs Enterobacteriaceae are shown in Table 5. A total of 81 isolates (72.32%) were resistant to ampicillin. Sensitivity to cephalosporins was ranged from 54.46% to 100%, while 94 (83.93%) isolates were sensitive to piperacillin-tazobactam. Ninety five

**Table 2.** Distribution of ESBLs Enterobacteriaceae with respect to clinical specimen.

| Bacterial isolates | Number of isolates | Specimens | | | |
|---|---|---|---|---|---|
| | | Blood | Wound and abscess | Urine | Respiratory secretion |
| ESBLs *Escherichia coli* | 20 | 2<br>(10%)*<br>(100%)** | 5<br>(25%)*<br>(83.33%)** | 12<br>(60%)*<br>(70.59%)** | 1<br>(5%)*<br>(33.33%)** |
| ESBLs *Klebsiella pneumoniae* | 8 | ND | 1<br>(12.5%)*<br>(16.67%)** | 5<br>(62.5%)*<br>(29.41%)** | 2<br>(25%)*<br>(66.67%)** |
| Total | 28 | 2 | 6 | 17 | 3 |

*% according to total number of the specified isolate; **% according to specimen type; ND, Not detected; ESBLs, extended spectrum-beta-lactamase.

(84.82%) isolates were sensitive to ciprofloxacin, while 100 isolates (89.29%) were sensitive to levofloxacin. All enterobacteriaceae were sensitive (100%) to carbapenem, amikacin and tigecycline.

In general, levels of multidrug resistance were higher among ESBLs producers than in non-ESBLs producers. ESBLs strains were highly resistant to most cephalosporins (>50%). ESBLs isolates were frequently resistant to ciprofloxacin (78.57%) and levofloxacin (71.43%). However, ESBLs producers were highly sensitive to amikacin (89.29%). All ESBLs (100%) isolates were still sensitive to tigecycline and carbapenem as shown in Table 6.

*Ps. aeruginosa* demonstrated resistance to several antibiotics (Table 7). *Ps. aeruginosa* were highly sensitive to carbapenem (90.48%), amikacin (90.48%), cefepime (80.95%), ceftazidime (76.19%), piperacillin (61.90%) and fluoroquinolones (80.95%). The other *Pseudomonas* sp. was sensitive to most tested agents.

The single *Stenotrophomonas maltophilia* isolate exhibited MDR and was susceptible only to trimethoprime/sulfamethoxazol, fluoroquinolones, and colistin.

*Acinetobacter baumannii* strains were resistant to most antibiotics tested (Table 8). Seven isolates out of 12 (58.33%) of *A. baumannii* were resistant to piperacillin/ tazobactam, ceftazidime, cefepime, carbapenem, and gentamicin while 8 isolates out of 12 (66.67%) were resistant to fluoroquinolones. Resistance to tigecycline and amikacin was 16.67% and 25%, respectively. All *Acinetobacter* spp. were sensitive to colistin (100%).

## DISCUSSION

In recent years, incidence of multidrug resistant pathogenic organisms has increased notably. Thus, therapeutic proposals have been modified according to the emergence of drug resistance and adapted to epidemiological markers of individual infectious processes, geographical variations of these markers and the availability of new antibacterial agents (Alvarez-Lerma et al., 2006).

In the present study, 176 Gram-negative bacilli isolates were obtained from 158 patients, and the ratio of males (41.14%) to females (58.86%) was similar to Al-Ghamdi et al. (2002). Other investigators reported a higher proportion of males (El-Amin and Faidah, 2012).

In our experience most patients were above 50 years (48.73%) which is comparable to previous reports from the same hospital (Madani, 2002) and other hospitals in Saudi Arabia (Asghar and Momenah, 2006; Khanfar et al., 2009). Results from this study corroborate the generally accepted rule that older patients are predisposed to infection.

The distribution of Saudi (32.28%) and non-Saudi (67.72%) observed here is similar to a previous report for the same hospital by Madani (2002). Studies in other Saudi hospitals however have indicated a higher proportion of Saudi patients (Asghar, 2006; Al-Anazi, 2009). Demographic data collected indicated that there was no selection in the terms of gender, nationality or age.

In our experience *E. coli* (38.07%), were most prevalent followed by *K. pneumoniae* (15.91%), *Ps. aeruginosa* (11.93%), *P. mirabilis* (9.66%) and *A. baumannii* (6.82%). Similar results have been previously reported by Kader et al. (2004).

In this study, GNB were detected most frequently from urine specimens (43.75%), which included *E. coli* (73.13%), *K. pneumoniae* (39.29%) and *A. baumannii* (41.67%). Other reports have similarly documented the highest number of GNB obtained from urine (Khanfar et al., 2009; Mansouri et al., 2012). In contrast, another study from Poland, reported that *A. baumannii* was predominantly isolated from blood samples (Wroblewska et al., 2007).

In this investigation, *Ps. aeruginosa* (52.38%) were cultured mostly from respiratory secretions other investigations have documented similar finding (Asghar

**Table 3.** Distribution of non-ESBLs Gram-negative bacilli with regard to wards.

| Bacterial isolates | Number of isolates | Wards | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Intensive care units | Medical | Pediatric | Surgical | Emergency room | Outpatient | Others*** |
| *Escherichia coli* | 47 | 5 (10.64%)* (12.82%)** | 4 (8.51%)* (14.81%)** | 5 (10.64%)* (50%)** | 7 (14.89%)* (35%)** | 6 (12.76%)* (46.15%)** | 18 (38.30%)* (66.67%)** | 2 (4.25%)* (16.67%)** |
| *Klebsiella pneumoniae* | 20 | 7 (35%)* (17.95%)** | 1 (5%)* (3.70%)** | 2 (10%)* (20%)** | 2 (10%)* (10%)** | 2 (10%)* (15.38%)** | 4 (20%)* (14.81%)** | 2 (10%)* (16.67%)** |
| *Klebsiella oxytoca* | 2 | 1 (50%)* (2.56%)** | ND | ND | ND | 1 (50%)* (7.69%)** | ND | ND |
| *Enterobacter aerogenes* | 2 | ND | 1 (50%)* (3.70%)** | ND | 1 (50%)* (5%)** | ND | ND | ND |
| *Enterobacter cloaceae* | 7 | 3 (42.86%)* (7.69%)** | 3 (42.86%)* (11.11%)** | ND | 1 (14.29%)* (5%)** | ND | ND | ND |
| *Citrobacter koseri* | 2 | ND | ND | ND | ND | 1 (50%)* (7.69%)** | 1 (50%)* (3.70%)** | ND |
| *Serratia marcescens* | 3 | 1 (33.33%)* (2.56%)** | ND | 1 (33.33%)* (10%)** | 1 (33.33%)* (5%)** | ND | ND | ND |
| *Serratia fonticola* | 1 | ND | 1 (100%)* (3.70%)** | ND | ND | ND | ND | ND |
| *Salmonella* sp. | 2 | 2 (100%)* (5.13%)** | ND | ND | ND | ND | ND | ND |

**Table 3.** Contd.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Pseudomonas aeruginosa* | 21 | 7 (33.33%)* (17.95%)** | 6 (28.57%)* (22.22%)** | ND | 1 (4.76%)* (5%)** | 1 (4.76%)* (7.69%)** | 3 (14.29%)* (11.11%)** | 3 (14.29%)* (25%)** |
| *Pseudomonas stutzeri* | 1 | ND | ND | ND | ND | 1 (100%)* (7.69%)** | ND | ND |
| *Stenotrophomonas maltophilia* | 1 | ND | ND | ND | ND | ND | ND | 1 (100%)* (8.33%)** |
| *Acinetobacter baumannii* | 12 | 7 (58.33%)* (17.95%)** | 2 (16.67%)* (7.41%) | 1 (8.33%)* (10%)** | ND | ND | 1 (8.33%)* (3.70%)** | 1 (8.33%)* (8.33%)** |
| *Acinetobacter junii* | 1 | 1 (100%) (2.56%)** | ND | ND | ND | ND | ND | ND |
| *Proteus mirabilis* | 17 | 3 (17.65%) (7.69%) | 7 (41.18%) (25.93%) | ND | 5 (29.41%)* (25%)** | 1 (5.88%)* (7.69%)** | ND | 1 (5.88%)* (8.33%)** |
| *Proteus vulgaris* | 5 | ND | 2 (40%) (7.41%) | ND | 1 (20%)* (5%)** | ND | ND | 2 (40%)* (16.67%)** |
| *Morganella morganii* | 4 | 2 (50%) (5.13%) | ND | 1 (25%)* (10%)** | 1 (25%)* (5%)** | ND | ND | ND |
| Total | **148** | **39** | **27** | **10** | **20** | **13** | **27** | **12** |

*% according to total number of specified isolates; ** % according to hospital wards, ***Other wards such as private and isolation; ND, Not detected.

and Faidah, 2009; Al-Ahmady and Mohamed, 2013).

During the course of this work, most GNB (26.70%) were obtained from ICUs patients. Similarly ICUs patients were frequently reported in other studies (Abdel-Fatah, 2005; Hadadi et al., 2008). In addition it was noted that although non-ESBLs *E. coli* (38.30%) isolates were recovered from out patients (obstetrics/gynecology clinics), 25% of ESBLs *E. coli* were mainly detected from ICUs patients. *K. pneumoniae* (35.71%), *Ps. aeruginosa* (33.33%) and *A. baumannii* (58.33%)

**Table 4.** Distribution of ESBLs Enterobacteriaceae with regard towards.

| Bacterial isolates | Number of isolates | Wards | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Intensive care units | Medical | Pediatric | Surgical | Emergency room | Outpatient | Others*** |
| ESBLs *Escherichia coli* | 20 | 5 (25%)* (62.5%)** | 3 (15%)* (100%)** | 2 (10%)* (50%)** | 3 (15%)* (60%)** | 5 (25%)* (100%)** | 1 (5%)* (100%)** | 1 (5%)* (50%)** |
| ESBLs *Klebsiella pneumoniae* | 8 | 3 (37.5%)* (37.5%)** | ND | 2 (25%)* (50%)** | 2 (25%)* (40%)** | ND | ND | 1 (12.5%)* (50%)** |
| Total | 28 | 8 | **3** | 4 | 5 | **5** | **1** | 2 |

*% according to total number of specified isolates; **% according to hospital wards, ***other wards such as private and isolation; ND, not detected. ESBLs: Extended spectrum-beta-lactamase.

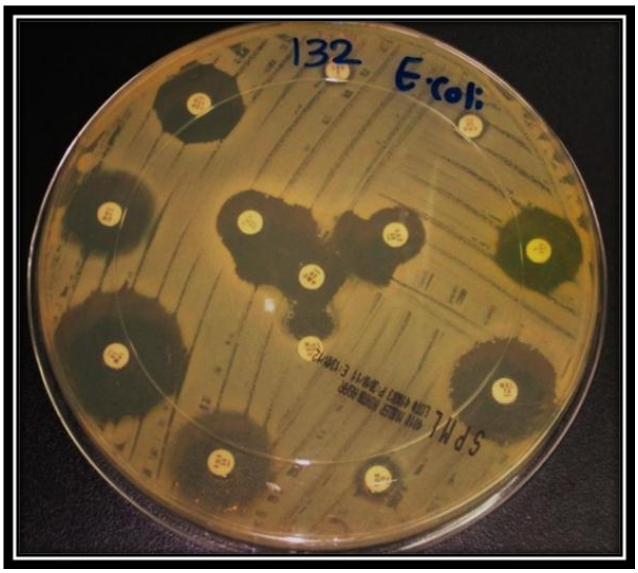


**Figure 2.** ESBLs producing *E. coli*, double disc synergy test. Showing enhanced zone of inhibition around more than one of the β-lactam-containing discs towards the clavulanic acid-containing disc.

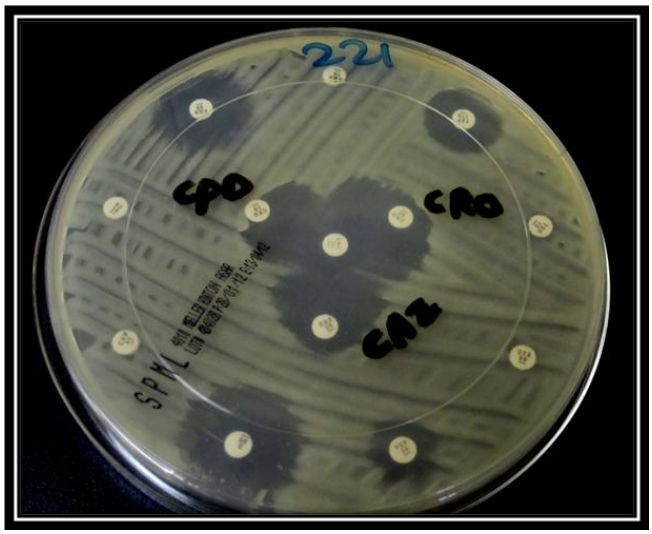


**Figure 3.** ESBLs producing *K. pneumoniae*, double disc synergy test.

were also associated most frequently with ICUs patients. Similar results were reported previously in Saudi Arabia (Asghar and Faidah, 2009; Al-Ahmady and Mohamed, 2013).

Other work has previously shown that ICU patients are implicated most frequently in infections with other micro-organisms also (Helmi et al., 2013). The incidence of multidrug resistant GNB among ICU patients may be due to prior antibiotic use, long antibiotics exposure, inadequate antibiotic therapy, or other reasons.

In our experience, ESBLs were rapidly identified by using DDST and Vitek 2 system. In addition to these methods, other studies have used E-test, combined discs or PCR methods for detecting ESBLs in GNB (Khanfar et al., 2009; Al-Sweih et al., 2011; Grover et al., 2013). Single disc diffusion method was not suitable for detecting ESBLs (Babay, 2002). However, further confirmatory tests are needed to confirm the presence of ESBL enzymes in such isolates.

In general, the results demonstrated that GNB have high rates of resistance to commonly used antibiotics. Attention should be paid to the use of ampicillin which shows high levels of resistance (> 50%).

For non-ESBLs Enterobacteriaceae, sensitivity to cephalosporins ranged from 54.46% to 100%, which is similar to another study (Al-Yaqoubi and Elhag, 2008).

Tazobactam in combination with piperacillin has excellent clinical efficacy in various infections and is a promising beta-lactamase inhibitor which has its own antibiotic activity (Niki, 2001). Resistance rate to piperacillin-tazobactam (16.07%) ciprofloxacin (13.39%)

**Table 5.** Susceptibility of 112 isolates of non-ESBLs Enterobacteriaceae to antimicrobial agents.

| Antibiotics | Disc Diffusion | | | MIC Vitek 2 | | | |
|---|---|---|---|---|---|---|---|
| | Number of isolates (%) | | | | Number of isolates (%) | | |
| | S | I | R | Range (µg/ml) | S | I | R |
| Ampicillin (AMP) | 30 (26.79) | 1 (0.89) | 81 (72.32) | ≤2 - ≥32 | 30 (26.79) | 1 (0.89) | 81 (72.32) |
| Piperacillin (PRL) | 58 (51.79) | 2 (1.79) | 52 (46.43) | ≤4- ≥128 | 47 (41.96) | 2 (1.79) | 63 (56.25) |
| Piperacillin-tazobactam (TZP) | 94 (83.93) | - | 18 (16.07) | ≤4- ≥128 | 88 (78.57) | 4 (3.57) | 20 (17.86) |
| Cephalothin (KF) | 61 (54.46) | 6 (5.36) | 45 (40.18) | ≤2 - ≥64 | 61 (54.46) | 6 (5.36) | 45 (40.18) |
| Cefoxitin (FOX) | 95 (84.82) | 1 (0.89) | 16 (14.29) | ≤4 - ≥64 | 84 (75) | 4 (3.57) | 24 (21.43) |
| Cefotaxime (CTX) | 94 (83.93) | 7 (6.25) | 11 (9.82) | ≤1- ≥64 | 94 (83.93) | 7 (6.25) | 11 (9.82) |
| Ceftazidime (CAZ) | 102 (91.07) | 3 (2.68) | 7 (6.25) | ≤1- ≥64 | 102 (91.07) | 3 (2.68) | 7 (6.25) |
| Cefepime (FEB) | 112 (100) | - | - | ≤1-8 | 112 (100) | - | - |
| Meropenem (MEM) | 112 (100) | - | - | ≤0.25-1 | 112 (100) | - | - |
| Imipenem (IPM) | 112 (100) | - | - | ≤1 | 112 (100) | - | - |
| Amikacin (AK) | 112 (100) | - | - | ≤2 -16 | 109 (97.32) | 3 (2.68) | - |
| Gentamicin (CN) | 102 (91.07) | - | 10 (8.93) | ≤1- ≥16 | 101 (90.18) | 1 (0.89) | 10 (8.93) |
| Tigycycline (TGC) | 112 (100) | - | - | ≤0.5-4 | 112 (100) | - | - |
| Ciprofloxacin (CIP) | 95 (84.82) | 2 (1.79) | 15 (13.39) | ≤0.25- ≥4 | 95 (84.82) | 2 (1.79) | 15 (13.39) |
| Levofloxacin (LEV) | 100 (89.29) | - | 12 (10.71) | ≤0.12- ≥8 | 100 (89.29) | - | 12 (10.71) |
| Nitrofurantoin (F) | 75 (66.96) | 2 (1.79) | 35 (31.25) | ≤16- ≥256 | 58 (51.79) | 19 (16.96) | 35 (31.25) |
| Trimethoprime/ sulfamethoxazole (SXT) | 85 (75.89) | - | 27 (24.11) | ≤20- ≥320 | 85 (75.89) | - | 27 (24.11) |

S, Sensitive; I, intermediate; R, resistant; MIC, minimum inhibitory concentration.

**Table 6.** Susceptibility of 28 isolates of ESBLs Enterobacteriaceae to antimicrobial agents.

| Antibiotics | Disc diffusion | | | MIC Vitek 2 | | | |
|---|---|---|---|---|---|---|---|
| | Number of isolates (%) | | | | Number of isolates (%) | | |
| | S | I | R | Range (µg/ml) | S | I | R |
| Ampicillin (AMP) | - | - | 28 (100) | 32 | - | - | 28 (100) |
| Piperacillin (PRL) | - | - | 28 (100) | ≥ 128 | - | - | 28 (100) |
| Piperacillin-azobactam (TZP) | 15 (53.75) | 2 (7.14) | 11 (39.29) | ≤4- ≥128 | 14 (50) | 3 (10.71) | 11(39.29) |
| Cefoxitin (FOX) | 20 (71.43) | - | 8 (28.57) | ≤4- ≥ 64 | 18 (64.29) | 2 (7.14) | 8 (28.57) |
| Cefotaxime (CTX) | - | - | 28 (100) | ≥64 | - | - | 28 (100) |
| Ceftazidime (CAZ) | | 3 (10.71) | 25 (89.29) | ≤1 - ≥64 | - | 3 (10.71) | 25 (89.29) |
| Cefepime (FEB) | 4 (14.29) | 8 (28.57) | 16 (57.14) | ≤1 - ≥64 | 4 (14.29) | 8 (28.57) | 16 (57.14) |
| Meropenem (MEM) | 28 (100) | - | - | ≤0.25 | 28 (100) | - | - |
| Imipenem (IPM) | 28 (100) | - | - | - | - | - | - |
| Amikacin (AK) | 25 (89.29) | 2 (7.14) | 1 (3.57) | ≤1 - ≥64 | 18 (64.29) | 9 (32.14) | 1 (3.57) |
| Gentamicin (CN) | 16 (57.14) | - | 12 (42.86) | ≤1 - ≥16 | 16 (57.14) | - | 12 (42.86) |
| Tigycycline (TGC) | 28 (100) | - | - | ≤0.5-1 | 28 (100) | - | - |
| Ciprofloxacin (CIP) | 6 (21.43) | - | 22 (78.57) | ≤0.25-≥4 | 6 (21.43) | - | 22 (78.57) |
| Levofloxacin (LEV) | 8 (28.57) | - | 20 (71.43) | ≤0.12-≥8 | 8 (28.57) | - | 20 (71.43) |
| Nitrofurantoin (F) | 24 (85.71) | - | 4 (14.29) | ≤16- 256 | 18 (64.29) | 6 (21.43) | 4 (14.29) |
| Trimethoprime/ sulfamethoxazole (SXT) | 9 (32.14) | - | 19 (67.86) | ≤20-≥320 | 9 (32.14) | - | 19 (67.86) |

S, Sensitive; I, intermediate; R, resistant; MIC, minimum inhibitory concentration.

**Table 7.** Susceptibility of 21 isolates of *Pseudomonas aeruginosa* to antimicrobial agents.

| Antibiotics | Disc Diffusion | | | MIC Vitek 2 | | | |
|---|---|---|---|---|---|---|---|
| | Number of isolates (%) | | | | Number of isolates (%) | | |
| | S | I | R | Range (µg/ml) | S | I | R |
| Ampicillin (AMP) | - | - | 21 (100) | ≥32 | - | - | 21 (100) |
| Piperacillin (PRL) | 13 (61.90) | - | 8 (38.09) | ≤4-≥128 | 7 (33.33) | 6 (28.57) | 8 (38.09) |
| Piperacillin-tazobactam (TZP) | 13 (61.90) | - | 8 (38.09) | ≤4-≥128 | 13 (61.90) | - | 8 (38.09) |
| Cefotaxime (CTX) | 1 (4.76) | - | 20 (95.24) | - | 1 (4.76) | - | 20 (95.24) |
| Ceftazidime (CAZ) | 16 (76.19) | 1 (4.76) | 4 (19.05) | ≤1-≥64 | 16 (76.19) | 1 (4.76) | 4 (19.05) |
| Cefepime (FEB) | 17 (80.95) | - | 4 (19.05) | - | 15 (71.43) | 2 (9.52) | 4 (19.05) |
| Meropenem (MEM) | 19 (90.48) | - | 2 (9.52) | ≤0.25-≥16 | 19 (90.48) | - | 2 (9.52) |
| Imipenem (IPM) | 19 (90.48) | - | 2 (9.52) | ≤1-≥16 | 19 (90.48) | - | 2 (9.52) |
| Amikacin (AK) | 19 (90.48) | - | 2 (9.52) | ≤2-≥64 | 19 (90.48) | - | 2 (9.52) |
| Gentamicin (CN) | 18 (85.71) | - | 3 (14.29) | ≤1-≥16 | 16 (76.19) | 2 (9.52) | 3 (14.29) |
| Tigycycline (TGC) | - | - | 21 (100) | ≤0.5-≥8 | - | - | 21 (100) |
| Ciprofloxacin (CIP) | 17 (80.95) | - | 4 (19.05) | ≤0.25-≥4 | 15 (71.43) | 2 (9.52) | 4 (19.05) |
| Levofloxacin (LEV) | 17 (80.95) | - | 4 (19.05) | ≤0.12-≥8 | 17 (80.95) | - | 4 (19.05) |
| Trimethoprime/ sulfamethox-azole (SXT) | - | - | 21 (100) | ≤20-≥320 | - | - | 21 (100) |

S, Sensitive; I, intermediate; R, resistant; MIC, minimum inhibitory concentration.

**Table 8.** Susceptibility of 12 isolates of *Acinetobacter baumannii* to antimicrobial agents.

| Antibiotics | Disc Diffusion | | | MIC Vitek 2 | | | |
|---|---|---|---|---|---|---|---|
| | Number of isolates (%) | | | | Number of isolates (%) | | |
| | S | I | R | Range (µg/ml) | S | I | R |
| Ampicillin (AMP) | - | - | 12 (100) | ≤2-≥32 | - | - | 12 (100) |
| Piperacillin (PRL) | 4 (33.33) | - | 8 (66.67) | ≤8-≥128 | 4 (33.33) | - | 8 (66.67) |
| Piperacillin-tazobactam (TZP) | 5(46.15) | - | 7 (53.85) | ≤4-≥128 | 4 (33.33) | 1 (8.33) | 7 (58.33) |
| Cefotaxime (CTX) | - | - | 12 (100) | ≤1-≥64 | - | - | 12 (100) |
| Ceftazidime (CAZ) | 5(41.67) | - | 7 (58.33) | 4-≥64 | 5 (41.67) | - | 7 (58.33) |
| Cefepime (FEB) | 5 (41.67) | - | 7 (58.33) | ≤1-≥64 | 5 (41.67) | - | 7 (58.33) |
| Meropenem (MEM) | 5 (41.67) | - | 7 (58.33) | - | - | - | - |
| Imipenem (IPM) | 5 (41.67) | - | 7 (58.33) | ≤1-≥16 | 5 (41.67) | - | 7 (58.33) |
| Amikacin (AK) | 8 (66.67) | - | 4 (33.33) | ≤2-≥64 | 8 (66.67) | - | 4 (33.33) |
| Gentamicin (CN) | 5 (41.67) | - | 7 (58.33) | ≤1-≥16 | 5 (41.67) | - | 7 (58.33) |
| Tigycycline (TGC) | 10 (83.33) | - | 2 (16.67) | ≤0.5-≥8 | 10 (83.33) | - | 2 (16.67) |
| Ciprofloxacin (CIP) | 4 (33.33) | - | 8 (66.67) | ≤0.25-≥4 | 4 (33.33) | - | 8 (66.67) |
| Levofloxacin (LEV) | 4 (33.33) | - | 8 (66.67) | ≤0.12-≥8 | 4(33.33) | - | 8 (66.67) |
| Colistin (CT) | 12 (100) | - | - | NA | NA | NA | NA |
| Nitrofurantoin (F) | - | - | 12 (100%) | ≥512 | - | - | 12 (100) |
| Trimethoprime/ sulfamethoxazole (SXT) | 5 (41.67) | - | 7 (58.33) | ≤20-≥320 | 5 (41.67) | - | 7 (58.33) |

S, Sensitive; I, intermediate; R, resistant; MIC, minimum inhibitory concentration.

and levofloxacin (10.71%) were documented. In comparison other studies in Saudi Arabia and globally have reported varying levels of resistance (Asghar and Faidah, 2009; Iqbal et al., 2014; Nivas et al., 2014).

Beta-lactam drugs are commonly included in the empirical antibiotics treatment of Gram-negative bacteria; however ESBLs producing bacteria may not be susceptible to such treatment. ESBLs prevalence shows wide variation from country to country and within the same country over a period of time but is generally on the increase (Yezli et al., 2014). This study shows that 20% (28/140) of Enterobacteriaceae were ESBLs producing strains including 20 isolates of *E. coli* and 8 isolates of *K. pneumoniae*. This is higher than earlier reports in Saudi Arabia by Kader and Kumar (4.8%) in 2004, and Khanfar et al. (6%) in 2009 but lower than those reported by Babay (36%) in 2002. Other countries have also shown variation, within the Arabian Gulf region, high ESBLs prevalence of 31.7% in Kuwait and 41% in the United Arab Emirates have been reported (Al-Zarouni et al., 2008; Mokaddas et al., 2008).

ESBLs producing strains were resistant to most commonly used antibiotic such as ampicillin (100%) and ciprofloxacin (78.57%) which is higher than reported in Bangladesh by Mowla et al. (2011). Khanfar et al. (2009) reported similarly high rates of resistance to ciprofloxacin (80%) in ESBL producers isolated from the eastern province of Saudi Arabia, but other studies found much lower rates in ESBL *K. pneumoniae* from Al-Qassim (9.1%) and Riyadh (11%), both in Saudi Arabia (Tawfik et al., 2011; Al-Agamy et al., 2009).

In this study, the carbapenems, tigecycline and amikacin were the most active agents against MDR Enterobacteriaceae such as *E. coli*, *K. pneumoniae*, *Ent. aerogenes, Ent. cloacae*, *S. marcescens*, and highly active against the ESBLs producing isolates. Similar results were reported in other studies (Kader and Kumar, 2004; Khanfar et al., 2009; Iqbal et al., 2014).

It has also been reported that the susceptibility of Enterobacteriaceae to the carbapenems was unaffected by the production of ESBLs (Turner, 2005). This could explain the high sensitivity of Enterobacteriaceae to carbapenems in this study. However, as reduced carbapenems susceptibility in ESBLs producing strains has been documented in Saudi Arabia (Balkhy et al., 2012) and in other countries (Jamal et al., 2011; El-Herte et al., 2012), hospitals should have proper policies and guidelines for the prudent use of antimicrobial agents and adequate infection control in order to avoid the emergence of further resistant isolates.

Our findings of high levels of resistance to non-beta lactam classes of antibiotics in ESBLs producing organisms are in agreement with other reports. Often attributed to self-transmissible R- plasmids are implicated (Shibl et al., 2012).

On the other hand, *Ps. aeruginosa* demonstrated high resistance rate to several antibiotics. During this survey, imipenem and amikacin show a higher susceptibility (90.48%) against *Ps. aeruginosa* followed by fluoroquinolones and cefepime (80.95% for each) then piperacillin-tazobactam (61.90%). This is similar to a previous study done in the same hospital (Eltahawy and Khalaf, 2001) as well as other hospitals in Saudi Arabia (Asghar, 2006; Memish et al., 2012; Al-Ahmady and Mohamed, 2013) and other countries (Al-Yaqoubi and Elhag, 2008; Benachinmardi et al., 2014). Another study, showed a lower susceptibility of *Ps. aeruginosa* to imipenem and amikacin compared to our observation (Jamshidi et al., 2009)

Our work shows that *A. baumannii* was MDR to a variety of antibiotics, including ampicillins, cephalosporins, carbapenem, fluoroquinolones and gentamicin confirming earlier reports in KSA (Memish et al., 2012; Al-Ahmadey and Mohamed, 2013; Al Masoudi et al., 2013). All *Acinetobacter* spp. were sensitive to colistin (100%) similar finding has been documented by Asaad et al. (2013).

This study shows that the single *Stenotrophomonas maltophilia,* was resistant to all antibiotics tested except trimethoprime/sulfamethoxazol, fluoroquinolones and colistin. Other studies showed similar results (Nicodemo and Garcia Paez, 2007; Asaad et al., 2013).

## Conclusion

Emerging antimicrobial resistance is currently the main concern of the medical community, because such resistant bacteria are becoming more difficult to treat. Our data showed a high rate of resistance among GNB but carbapenems are still effective against these organisms. However, MDR is common with *Ps. aeruginosa* and *A. baumannii*. Strict infection control measures, formulation of antibiotic policy and continual monitoring and surveillance is required globally. New antimicrobial agents and vaccines must be developed. Further studies are recommended to evaluate both antimicrobial susceptibility of GNB isolated and their mechanism of resistance in different hospitals of Saudi Arabia and other countries.

## Conflict of interests

The authors did not declare any conflict of interest.

## ACKNOWLEDGEMENTS

The authors thank King Abdulaziz City for Science and Technology, Saudi Arabia for funding this study. Authors were also thankful for clinical microbiology laboratory staff at King Abdulaziz University Hospital, Jeddah, Saudi Arabia for their cooperation with respect to the Vitek 2 system.

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