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Assessment of antimicrobial resistance patterns in *Escherichia coli* isolated from clinical samples in Madinah, Saudi Arabia

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Escherichia coli **is a Gram-negative bacteria that causes various diseases, including pneumonia, urinary tract infections, and diarrhoea. The present work is an effort to study antimicrobial resistance pattern in this bacteria. Clinical samples (6840) were collected from King Fahd Hospital in Madinah, Saudi Arabia and screened for *E. coli* strains. Of all positive samples obtained from different clinical sources, about 3% isolates represented *E. coli* and 71.6% of these were collected from clinical samples of male patients. *E. coli* isolates were recovered from sputum (20.58%), wound (55.88%), and catheters tips (9.31%) representing about 86% of all clinical specimens. Antimicrobial susceptibility pattern of the *E. coli* isolates to twelve different antimicrobials revealed that all the isolates (100%) were susceptible to imipenem, amikacin, and aztreonam. Cefoxitin, ceftazidime and ciprofloxacin showed a sensitivity of 98.7%. This was followed by gentamycin (98.6%), piperacillin (95.7%), cotrimoxazole (92.3%), cephalothin (91.4%), and Augmentin (91.1%). Ampicillin showed the least susceptibility of 87.5%. Hence, cotrimoxazole, Augmentin, and ampicillin should be prescribed with care in order to avoid increasing resistance patterns in *E. coli*. Results also demonstrate that frequency of *E. coli* infections was highest during summer and winter seasons representing about 30% each. Autumn season, which coincided with the season of pilgrimage, recorded about 22% of infections while spring season had the least recorded percentage with only 17% of infections. This study is therefore a step towards the generation of national data on the prevalence of antimicrobial resistance patterns of *E. coli*.**

Key words: *Escherichia coli,* antimicrobial susceptibility pattern, multi-drug-resistance, antimicrobials.

INTRODUCTION

Multidrug resistant (MDR) has become a public health issue which is estimated to cause maximum deaths by the year 2050 along with increasingly high health expenses. Besides, not many effective drugs are available for the treatment of multidrug-resistant Gram-negative bacteria (Prestinaci et al., 2015; Alawi and Darvesh, 2016). Recent reports from Middle East including Saudi Arabia, show a considerable and increasing prevalence of antimicrobial-resistant bacteria (Alawi and Darvesh, 2016; Zowawi, 2016). It has also been reported that although many physicians are aware of the dangers of MDR, majority of them do not comply with antimicrobial prescribing guidelines (Baadani *et* al., 2015; Al-Harthi et al., 2015).

Gram-negative bacteria, specifically those belonging to the family Enterobacteriaceae, can acquire genes that encode for multiple antimicrobial resistance mechanisms, including extended-spectrum-lactamases (ESBLs), AmpC-β-lactamase, and carbapenemases (Bader et al., 2017). One member of this group, *Escherichia coli* (*E. coli*) is ubiquitous and is present in both animals and the environment (Guenther et al., 2011). This gram-negative, facultatively anaerobic, rod-shaped, coliform bacteria is also the most common cause of food and water-borne human diarrhea worldwide, causing many deaths especially in young children (Hunter et al., 2003). It is the leading cause of urinary tract infections (UTIs), blood stream infections, wounds infections, otitis media and other complications in humans (Prestinaci et al., 2015). More than 80% of UTIs occur in outpatients and *E. coli* accounts for more than 50% of the infections in these patients (Kirac et al., 2016). A rise in antimicrobial resistance has been reported in *E. coli* worldwide which is causing complications and treatment issues (Zowawi, 2016). A number of studies have been done in Kingdom of Saudi Arabia (KSA) on the antimicrobial resistance patterns of *E. coli* from various clinical sources (Halawani, 2011; Masoud et al., 2011; Zowawi, 2016). The present study is another effort to determine antimicrobial susceptibility of *E. coli* from clinical sources at a busy hospital at Madinah, KSA.

MATERIALS AND METHODS

Sample collection

Different clinical samples such as sputum, wound swab, bile, tracheal aspirate (Tr. asp.), throat aspirate (Th. asp.), catheter tip, pus, abdominal abscess (Abd. ab.), ear swab, peritoneal wound swab (Peri. w.s.), pleural fluid (Pler. fluid), vaginal swab (VS), urethral discharge (UD), eye cornea swab (ECS), bone tissues, brain tube were collected from 6840 patients suspected of bacterial infection at King Fahd Hospital at Madinah, KSA. Clinical samples were cultured to isolate the organisms. Demographic data such as sex of the patients was recorded prior to sample collection.

Culture and identification

The clinical samples were collected and aseptically inoculated on blood agar, chocolate agar, cystine-lactose-electrolyte-deficient (CLED) agar and MacConkey agar (Oxoid Cambridge, UK) according to Centers for Disease Control and Prevention Guidelines (CDCP, 2013). The culture plates were incubated at 37°C for 24 h. Identification was done based on morphological characteristics of the colonies including size, shape, colour, pigmentation and haemolytic nature.

Biochemical characterization

Suspected *Escherichia coli* colonies isolated were further identified through biochemical tests (Barrow and Felthan, 2003) using standard procedures and Phoenix automated microbiology 100 ID/AST system (Becton Dickinson Company, Sparks, Md). Identification included the following tests: Nitrate reduction test, citrate utilization test, oxidase test, H_2S gas production, methyl-red test, indole test, urease test, Voges-Proskauer test and lactose fermentation (Forbes et al., 2007).

Antimicrobial susceptibility test

Susceptibility to antimicrobial agents was determined by using the disk diffusion method (Oqunshe, 2006), and Phoenix automated microbiology 100 ID/AST system (Becton Dickinson Company, Sparks, Md.). The following antimicrobial agents obtained from BDH (London, UK) were used: Ampicillin (10 µg), Augmentin [amoxycillin + clavulinic acid (20/10 µg)], gentamycin (10 µg), cefoxitin (30 µg), cephalothin (30 µg), cotrimoxazole[trimethoprim-sulfamethoxazole 1:19 (25 µg)], amikacin (30 µg), ceftazidime (30 µg), aztreonam (30 µg), piperacillin (100 µg), imipenem (10 µg), and ciprofloxacin (5 µg). The inocula were prepared by growing the *E. coli* strains on separate agar plates and colonies from the plates were transferred with a loop into 3 ml of normal saline. The density of these suspensions was adjusted to 0.5 McFarland standards. The surface of Muller-Hinton agar (Oxoid Cambridge, UK) plate was evenly inoculated with the organisms using a sterile swab. The swab was dipped into the suspension and pressed against the side of the test tube to remove excess fluid. The wet swab was then used to inoculate the Muller-Hinton agar by evenly streaking across the surface. By means of a disc dispenser (Oxoid Cambridge, UK), the antimicrobial discs were applied onto the surface of the inoculated agar and the plates were incubated overnight at 37°C. The diameter of zone of growth inhibition observed was measured and compared to the chart provided by Clinical and Laboratory Standards Institute (CLSI, 2015).

RESULTS AND DISCUSSION

MDR is an alarming issue that is increasing continuously day by day; the main reason being inappropriate use and abuse of antimicrobials. Self-medication leads to patients consuming inadequate drug doses. MDR has to be monitored at several levels starting from basic research on how resistance develops in bacteria, to formulating strategies on regulating the dosage and susceptibility to different antimicrobials. When *E. coli* becomes resistant to carbapenems, like other bacteria of the Carbapenem-Resistant *Enterobacteriacae* (CRE) group, it becomes

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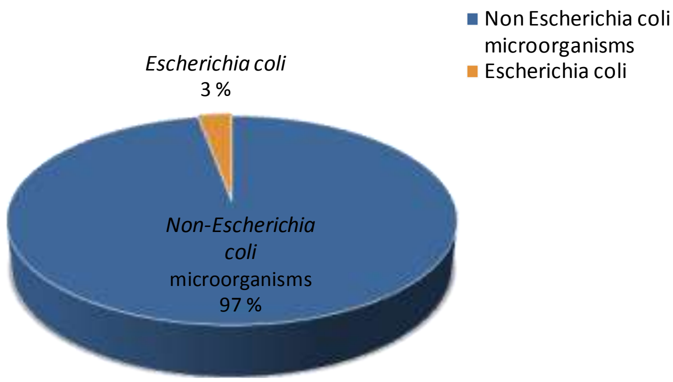

Figure 1. Percentage of *E. coli* specimens in comparison with other clinical isolates**.**

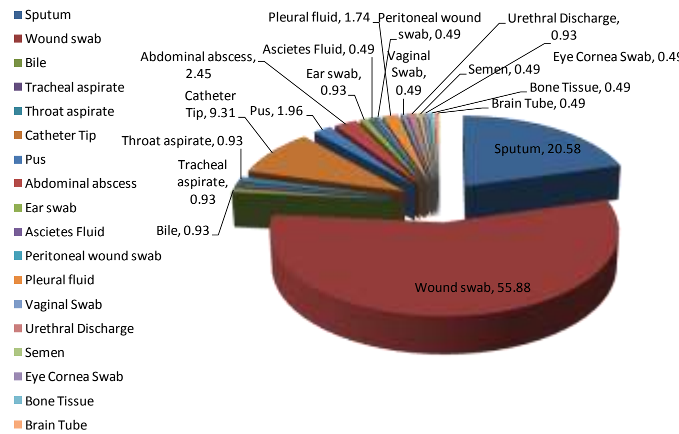

Figure 3. Percentage of *E. coli* specimens isolated from different clinical samples.

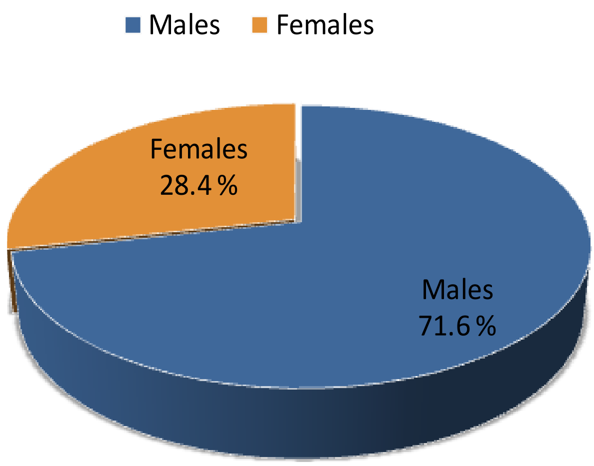

Figure 2. Percentage of isolates found positive for *E. coli* in males and females**.**

resistant to almost all available antimicrobials leading to many casualties each year (Ventola, 2015). Several studies have been reported on antimicrobial resistance patterns in *E. coli* from KSA (Rotimi et al., 1998; Al-Johani *et* al., 2010; Halawani, 2011; Zowawi, 2016) but none has been reported from Madinah, one of the two important cities visited by many pilgrims all year round. The present study is an attempt to study the antimicrobial resistance pattern of *E. coli* isolated from patients at King Fahad Hospital, Madinah, KSA. Exactly 6840 samples were collected from clinical sources over a period of 14 months and screened for *E. coli*. Results show that in comparison to other clinical isolates, only 3.0% *E. coli* strains were isolated (Figure 1). No *E. coli* isolate was recovered from some samples including urine, blood, ascetic fluid, nasal swabs, axilla, and perineum. Of the positive isolates, 71.6% were from clinical samples of male patients while 28.4% were from females (Figure 2). A similar study done in Makkah has also recorded a higher percentage of isolates from males (Haseeb et al., 2016).

Figure 3 shows the percentage of *E. coli* strains that could be retrieved from various sources. Majority of the *E. coli* strains were isolated from wound swabs (55.88%) and sputum samples (20.58%) followed by catheter tips (9.31%). While 2.45, 1.96 and 1.74% of *E. coli* isolates were recovered from abdominal abscess, pus and pleural fluid samples respectively. For the remaining clinical samples, less than 1% were recovered in bile, tracheal and throat aspirates, ear swabs, urethral discharge, ascites fluid, peritoneal wound swab, vaginal swabs, semen, eye cornea swabs, bone tissue and brain tube. Table 1 gives a gender-wise estimation of the number of male and female samples isolated from different sources. In wound swabs and sputum samples, 61.9 and 66.8%, respectively were obtained from males. Only 19 *E. coli* strains were isolated from catheter tips wherein 16 were from males and only 3 were from female patients. The male to female ratio in abdominal abscess, pus and pleural fluid were 4:1, 2:2 and 2:1, respectively. High isolation rates from sputum and wound specimens have been reported earlier also (Masoud et al., 2011; Kibret and Abera, 2011).

As described previously, the gender-wise distribution of the samples revealed that in general, greater number of *E. coli* strains were isolated from males which may be indicating that adult males are more susceptible to infection than adult females (Haseeb et al., 2016; Magliano et al., 2012). The results can be elucidated on the basis of different lifestyles and socio-economic conditions of the patients. Since the males constitute a larger workforce in Saudi Arabia, it is not surprising that greater samples were obtained from males than females. Only 1 sample each was available from ascites fluid, peritoneal wound swab, vaginal swabs, semen, eye cornea swabs, bone tissue and brain tube.

Antimicrobial drug susceptibility assay was performed

Table 1. Gender-wise distribution of *E. coli* specimens isolated from different sources.

	Source of specimens																
Sex	**Sp**	**WS**	**Bile**	**Tr**	**Th**	**Cath**	**Pus**	**Abd**	**Ear**	**AF**	**Peri**	**Pler**	**VS**	**UD**	**Semen**	**ECS**	**Bone tissue**	**Brain tube**
M	30 (66.8)	82 (61.9)	1 (50)	2 (40)	1 (66.7)	16 (82.1)	2 (50)	4 (80)	1 (50)	1 (100)	0 (0)	2 (66.7)	0 (0)	2 (100)	1 (100)	0 (0)	0 (0)	1 (100)
F	12 (33.2)	32 (38.1)	1 (50)	0 (60)	1 33.3)	3 (17.9)	2 (50)	1 (20)	1 (50)	0 (0)	1 (100)	1 (33.2)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)
Total	42	114	2	2	2	19	4	5	2	1	1	3	1	2	1	1	1	1

M, Males; F, Females; Sp, Sputum; WS, Wound swab; Tr, Tracheal aspirate; Th, Throat aspirate; Cath, Catheter Tip; Abd, Abdominal abscess; AF, Ascietes Fluid; Peri, Peritoneal wound swab; Pler, Pleural fluid; VS, Vaginal Swab; UD, Urethral Discharge; ECS, Eye Cornea Swab. Percentage (%) values are given in parentheses.

Table 2. Percentage (%) of antimicrobial sensitivity pattern of *E. coli* specimens to different antimicrobials.

Antibiotic	Sensitive	Resistant	Intermediate
Ampicillin	87.5	12.5	0.0
Augmentin	91.1	8.9	0.0
Gentamycin	98.6	1.4	0.0
Cefoxitin	98.7	1.3	0.0
Cephalothin	91.4	0.6	0.0
Cotrimoxazole	92.3	7.7	0.0
Amikacin	100.0	0.0	0.0
Ceftazidime	98.7	1.3	0.0
Aztreonam	100.0	0.0	0.0
Piperacillin	95.7	4.3	0.0
Imipenem	100.0	0.0	0.0
Ciprofloxacin	98.7	1.3	0.0

using antimicrobial discs of ampicillin, augmentin, gentamycin, cefoxitin, cephalothin, cotrimoxazole, amikacin, ceftazidime, aztreonam, piperacillin, imipenem and ciprofloxacin and the results are shown in Table 2. The antimicrobials imipenem, aztreonam and amikacin were the most effective drugs against *E. coli* strains with 100% sensitivity followed by ciprofloxacin, ceftazidime and cefoxitin with 98.7% sensitivity. Gentamycin was also effective with a sensitivity of 98.6%. Amikacin and aztreonam are treatment options for infections caused by bacteria belonging to carbapenem-resistant enterobacteriaceae (CRE) group (Bader et al., 2017). Imipenem has been highly effective against Gram-negative bacteria as shown by several other studies (Mohammed et al., 2016; Bahashwan and Shafey, 2013; Dash et al., 2014; Alam et al., 2017). Aztreonam and ciprofloxacin have been recommended as better antimicrobials against *E. coli* (Kirac et al., 2016). The percentage resistance to the antimicrobials used in the present study was not very high and was in the following sequence: Ampicillin (12.5 %) > Augmentin (8.9 %) > cotrimoxazole (7.7 %) > piperacillin (4.3 %) > gentamycin (1.4 %). Cefoxitin, ceftazidime and ciprofloxacin showed percentage resistance of 1.3% while amikacin, aztreonam and imipenem showed no resistance at all. The antimicrobial cephalothin showed a low resistance of 0.6% only. The results are

Table 3. Percentage (%) of *E. coli* infections pattern during different seasons.

Season	Percentage (%) of *E. coli* infections
Summer (22 June -22 September)	30.2
Autumn (23 September -21 December): Pilgrimage season	21.6
Winter (22 December -30 Mars)	30.9
Spring (21 Mars-21 June)	17.3

consistent and in compliance with previous studies (Inan and Gurler, 2004; Kirac et al., 2016). Attention should be given while prescribing cotrimoxazole, Augmentin, and ampicillin to avoid increasing resistance patterns by *E. coli.* They should be used in life threatening mutidrug resistant infections where there is no other alternative. In general, prescription for infection treatment should be based on WHO's critically important antimicrobials for human medicine.

Seasonal variations are commonly observed while studying the incidence of bacterial infections. These seasonal trends are influenced by several factors which can be identified by exploring their prevalence in detail (Fares, 2013). With the help of this and similar studies, novel and improved infection control strategies can be formulated. Several reports claim that bacterial infections always peak during summers and winters (Perencevich et al., 2008; Eber et al., 2011; Richet, 2012). Table 3 illuminates the *E. coli* infection pattern during four different seasons of the year in Madinah. Infections occurred with a higher and similar frequency in both summers (30.2%) and winters (30.9%). In the intermediate seasons, that is, autumn and spring, when the temperatures are not extreme, the percentage of *E. coli* infection reduces significantly to 21.6% in autumn and 17.3% in spring. During this period, the autumn season coincides with the annual pilgrimage called Hajj when a huge population of pilgrims visits this city. The reason for the decline in the percentage of infection may be due to the efforts of the health care workers in that period as the health authorities take special precautions in controlling and monitoring outbreaks of different microbes. A sudden rise in the percentage of infection cases after 21st June when summers start is not surprising. Similarly a sudden rise can be seen after 21th December when winters begin is also reported earlier (Richet, 2012). Similar patterns have also been observed with other gram negative bacterial species of *Proteus* (Bahashwan and Shafey, 2013), *Klebsiella* (Ghanem et al., 2017) and *Pseudomonas* (Saeed et al., 2018) during the same period of study.

Saudi Arabia has to face several challenges to keep both infections and MDR in control especially in the two holy cities. There is an influx of pilgrims throughout the year but it is during the time of the annual pilgrimage (Hajj), the cities are vulnerable to epidemics. But interestingly, during this season which coincides with autumn, increase in percentage of *E. coli* infection was not observed. Implementation of the World Health Organization (WHO) hand hygiene program and the Gulf Cooperation Council (GCC) Infection Control Program (Yezli *et* al., 2014) are some of the good initiatives taken by the Saudi government in controlling spread of resistant pathogens in healthcare units. Another program that helps in reducing MDR is the antimicrobial stewardship program (Alawi and Darwesh, 2016; Zowawi, 2016).

Conclusion

Wound swabs followed by the sputum samples turned out to be the largest source of *E. coli* isolates. Samples from male patients were greater in comparison to female patients, maybe because males are at a larger risk to infection. The antimicrobials imipenem, aztreonam and amikacin showed 100% sensitivity. These infections occurred with a higher frequency in both summers and winters but the infection percentage dropped during intermediate seasons. To limit the inappropriate use of antimicrobials and control the spread of MDR, there is a need of active surveillance, creating awareness in the medical community and changing the attitude and prescribing habits of physicians. New guidelines and awareness programs should be formulated and strictly followed. More and more studies should be done on MDR and sensitivity pattern of antimicrobials. Studies like this will help in developing better infection control policies and generate local databases for infection control strategies within this region.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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