The Relationship Between the Type of Infection and Antibiotic Resistance

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Ninety-one Bacterial samples were collected from Al-Sader Medical City in Al-Najaf province during a period of 11 weeks from 2-7-2016 to 15-9-2016. The isolates were identified according to cultural characteristics and biochemical activities. The results have revealed that 25 (27%) samples were *E.coli*, 22 (24%) samples were *Staphylococcus* spp, 27 (30%) samples were *Proteus* spp, and 17 (22%) samples were *Enterococcus* spp. The susceptibility of bacterial isolates to 22 antibiotics was tested using disc diffusion method. The results have revealed that in *E.coli* the most resistance was seen to Cotrimoxaz antibiotic with percentage of 67% . In *Staphylococcus* spp, most resistance was seen to Azactam antibiotic with percentage of 83% while susceptibility to Vancomycin was the most. In *Enterococcus* spp, the most resistance was seen to Vancomycin. *Proteus* spp, showed the most resistance against Ampicillin/cloxacillin. The distribution of bacterial infection among different genders and their effect on bacterial resistance were also tested. The results showed that infections with *E.coli* were 12 (48%) in males while in females 13 (52%). Most of *Staphylococcus* spp 13 (59%), has been isolated from males, while in females 9 (41%). Males infected with *Proteus* spp constitute 13 (48%), on the other hand, 14 (52%) were isolated from females. Infections with *Enterococcus* spp were distributed as 8 (47%) and 9 (53%) in males and females respectively. The different bacterial sites where also correlated and tested against the susceptibility results. All isolates displays different resistance to different antibiotics that varies with infection site and patient gender. In *E. coli* the resistance to antibiotics in UTI and intestinal disease was approximately the same. In *Staphylococcus spp.* and *Proteus spp.* wounds infections exhibit more resistant than other types of infections. In *Enterococcus spp.* UTI infections exhibit more resistant than wounds infections.

Keywords: Bacterial Infection, Antibiotic Resistance, Al-Najaf.

Antibiotics are produced naturally by some fungi and bacteria as a secondary metabolites to kill other microorganisms that live in the same habitat for the sake of nutritional competition especially in the environments of limited nutritional resources (Bennett and Feibelman, 2001). The antibiotics used to treat people nowadays are usually derived from these natural products (Clardy *et al.*, 2009). Antibiotics are used to treat many illnesses caused by bacterial infections, from ear and skin infections to pneumonia, food poisoning, meningitis, and other life-threatening infections so that antibiotics are essential tools for physicians at any given time.

Between 25% and 40% of hospital patients are receiving antibiotics intravenously (Equi *et al.*, 2002; Wilson, 2006). As antibiotic became widely used, resistant bacterial strains

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having the ability to inactivate the drug became prevalent, therefore, chemical and structural studies that deals with the antibiotic synthesis were undertaken to modify penicillin chemistry in order to prevent its enzymatic destruction by penicillinases (â-lactamases) which are produced by drug resistant bacteria (Gold and Moellering Jr, 1996; Kardos and Demain, 2011). Interestingly, the ability of bacteria to produce â-lactamases became possible by using the gene based molecular techniques (Al-Shamarti, 2010). This, however, is an appreciated prospect to provide resistance profile of pathogenic bacteria in order to determine the effective antibiotic to be used in treatment (D'costa *et al.*, 2006). Antibiotic resistance could arise as a result of spontaneous or induced genetic alteration in bacteria in addition to the horizontal gene transfer by means of conjugation among different bacterial cells. Thus, antibiotic resistance genes which had evolved as a result of natural selection could be shared (Mazodier and Davies, 1991). According to the concept of natural selection, random exposure to antibiotic puts evolutionary stress on bacteria to develop antibiotic resistance traits. Many antibiotic resistance genes are found on plasmids, enabling their transfer (Bennett, 2008). When a bacterium has the ability to resist several types of different antibiotic as a result of having several resistance genes, it is formally termed as multidrug resistant (MDR) or, informally, super bacterium or superbug (D'Costa *et al.*, 2011).

Aim of the study

The aim of this study is to correlate between the type of infection and antibiotic resistance. This aim is reached via surveying many infections caused by many bacteria, then performing antibiotic susceptibility test.

Methodology

Preparation of Culture media

Preparation of culture media was according to the instruction manual supplied with each medium.

Collection and Identification of Bacterial Samples

The samples were collected from the hospital and bridged directly to the laboratory for identification. The identification of bacterial samples was carried out depending on the cultural characteristics and biochemical tests according to (Mac Faddin, 2000).

Procedure for Performing the Disc Diffusion Test

Inoculum Preparation

Direct Colony Suspension Method

As a convenient alternative to the growth method, the inoculums were prepared by making a direct broth or saline suspension of isolated colonies selected from a 18- to 24-hour agar plate (a nonselective medium, such as blood agar or nutrient agar, has been used). The suspension is adjusted to match the 0.5 McFarland turbidity standard, using saline and a vortex mixer.

Inoculation of Test Plates

Optimally, within 15 minutes after adjusting the turbidity of the inoculums suspensions, a sterile cotton swab is dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This is necessary to remove excess inoculum from the swab.

The dried surface of a Mueller-Hinton agar plates was inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an equal distribution of inoculum. As a final step, the rim of the agar is swabbed. The plate lids were left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

Application of discs to inoculated agar plates

Antibiotics discs produced by bioanalysa company were used. The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc was pressed down to ensure complete contact with the agar surface. Then, the plates were inverted and placed in an incubator set to 37°C within 15 minutes after the discs were applied.

RESULTS AND DISCUSSION

The correlation of infection with the type of bacteria

The 91 Bacterial isolates from different infections obtained from Al-Sader medical city in

Al-Najaf province over the period from 2-7-2016 to 15-9-2016, were identified and correlated with the site of infection as shown below in table 1.

The identification of bacterial samples showed that most of the isolates (30%) were *Proteus* folled by *E. coli* (27%), *Staphylococcus* (24%) , and *Enterococcus* (19%).

The results showed that UTI is mainly caused by *E. coli*. This goes in agreement with (Nicolle, 2008) who showed that *E. coli* is the cause of 80–85% of urinary tract infections, with *Staphylococcus saprophyticus* as a causative agent in 5–10% of UTI. Most of wound infections has been shown to be caused by Staphylococcus in the present study. This agrees with (Toshkova *et al.*, 2001; Flora, 2002; Baggett *et al.*, 2004) in explaining that *Staphylococcus aureus*, is the common cause of wound infections.

Correlation between bacterial infections and gender

The type of infection has been correlated with the gender of the patient and with the type of bacteria as shown in table 2.

The results showed that the ratio of male to female patients is tend to be equal regardless the type of infections, but, when we consider the type of infection it is clear that males are significantly more affected by wound infections than females as 81.5% of wound infections appeared in mlase. This could be due to the outdoor working environment of the males which makes more exposed to have injuries than females and, consequently, having wound infections more frequently than females. On the other hand, the results showed that UTI is more frequent in females than in males as 70% of UTI appeared in urine samples taken from females. This is most likely due to the anatomy of the female urethra and genital area which makes female more vulnerable to have UTI than males. This agrees with the findings that has been shown previously that UTI is more common in females (Stamm, 1991; Foxman *et al.*, 2000; Harrington and Hooton, 2000; Chittagong, 2011).

Correlation between the type of infection and antibiotic resistance in *E.coli*

E. coli was isolated only from urine and stool as a causative agent of UTI and intestinal infection. The antibiotic resistance against 13 different antibiotics was evaluated and correlated to the type of infection which are UTI and intestinal infections. The resistance percentage for each antibiotic was calculated, then the mean of resistance percentages was calculated for both types of the previously mentioned infections (Table

Table 1. Distribution of bacteria and infections

Infection Bacteria	Wound	UTI	Otitis media	Intestinal Disease	Total	Percentage
E.coli	0	11	0	14	25	27%
Staphylococcus	10	7	5	0	22	24%
Proteus	9	10	8	0	27	30%
Enterococcus	8	9	0	0	17	19%
total	27	37	13	14	91	100%

Table 2. Correlation between bacterial infections and patient gender

Bacteria	*E.coli*		*Staphylococci*		*Proteus*		*Enterococci*		Total	
Infection	male	female	male	female	male	female	male	female	male	female
Wound	-	-	8	2	8	1	6	2	22	5
UTI	4	7	2	5	3	7	2	7	11	26
Otitis media	-	-	3	2	2	6	-	-	5	8
Intestinal Disease	8	6	-	-	-	-	-	-	8	6
Total & percentage	12 48%	13 52%	13 59%	9 41%	13 48%	14 52%	8 47%	9 53%	46 50.5%	45 49.5%

Table 3. Correlation between the type of infection and antibiotic resistance in *E. coli*:

Infection \ Antibiotic		Ampicillin 61%			Gentamycin 29%			Amikacin 33%			Cotrimoxazole 67%			Chloramphenic-ol 10%		
		s	i	r	s	i	r	s	i	r	s	i	r	s	i	r
UTI	NO	4	-	7	6	-	5	7	-	4	5	-	6	9	-	2
	%	36%		64%	54%		46%	64%		36%	46%		54%	88%		12%
Intestinal infection	NO	6	-	8	12	-	2	10	-	4	3	-	11	13	-	1
	%	43%		57%	88%		12%	71%		29%	21%		79%	93%		7%

Infection \ Antibiotic		Nitrofurantoin 36%			Ceftazidime 24%			Ceftriaxone 29%			Amoxyclav 31%		
		s	i	r	s	i	r	s	i	r	s	i	r
UTI	NO	8	-	3	6	3	2	7	-	4	7	2	2
	%	72%		28%	55%	33%	12%	64%		36%	64%	24%	12%
Intestinal infection	NO	8	-	6	9	-	5	11	-	3	7	-	7
	%	57%		43%	64%		36%	79%		21%	50%		50%

Infection \ Antibiotic		Nalidixic Acid 45%			Ciprofloxacin 45%			Cephalothin 29%			Ampicillin/cloxacillin 54%		
		s	i	r	s	i	r	s	i	r	s	i	r
UTI	NO	6	-	5	5	-	6	6	-	5	7	-	4
	%	54%		46%	46%		54%	54%		46%	64%		36%
Intestinal infection	NO	8	-	6	9	-	5	12	-	2	4	-	10
	%	57%		43%	64%		36%	88%		12%	29%		71%

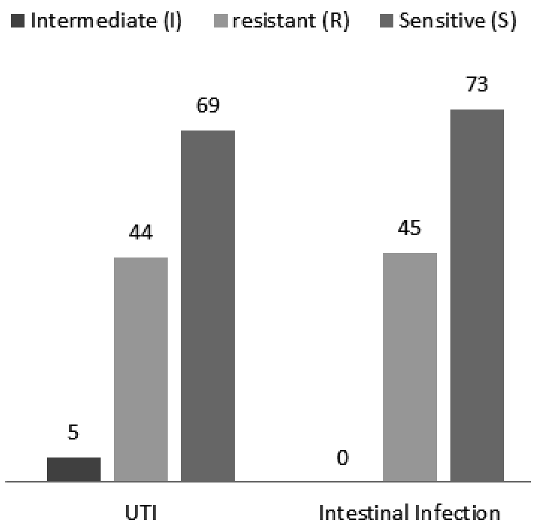

Fig. 1. The type of infection and antibiotic resistance in *E. coli*

3). The results showed approximate similarity of antibiotic resistance in both UTI and intestinal infections (Figure1).

The most resistance was seen to ampicillin with a percentage of 64% in UTI. This agrees with (Chittagong, 2011) who showed that the most resistance rates for *E. coli* detected from urine culture were Ampicillin, Doxycycline, Cephalexin, Cephradine, Cotrimoxazole, Cefixime, Ceftroxone and Ciprofloxacin except for Cefuraoxime to which *E. coli* was significantly sensitive. The antibiotic resistance pattern by *E.coli* has been shown to be 40% for Amoxicillin, 23% for Cephradine, 21% for Cotrimoxazole, 6% for Doxycycline, 4% for Cefixime, 2% for Cephalexin, 2% for Ceftroxone and 2% for Ciprofloxacin (Bhowmick and Rashid, 2004; Mohammadi *et al.*, 2010; Manikandan *et al.*, 2011)**.** (Omoregie *et al.*, 2011) showed that 60% of *E. coli* isolates were susceptible to Gentamycin.

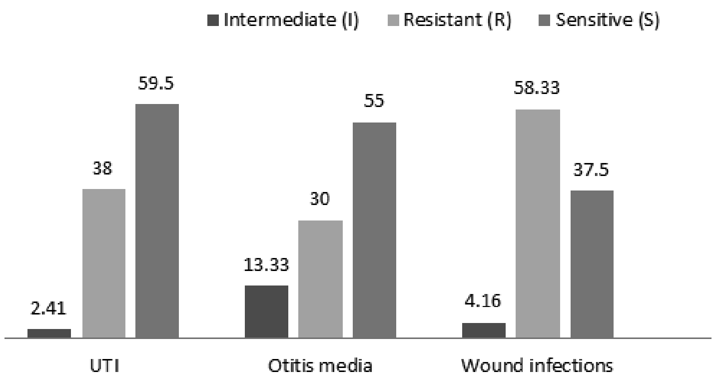

Fig. 2. The type of infection and antibiotic resistance in *Staphylococcus* spp

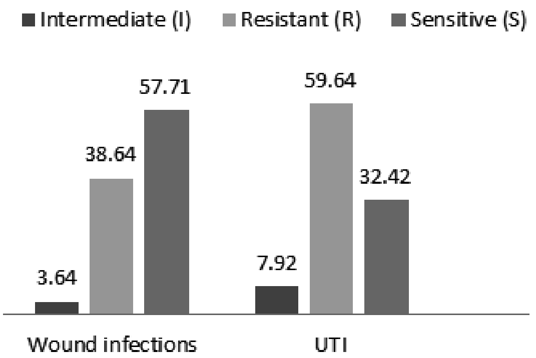

Fig. 3. The type of infection and antibiotic resistance in *Enterococcus* spp.

Correlation between the type of infection and antibiotic resistance in Staphylococcus spp.

Staphylococcus isolated from wound infections has been shown to be more resistant against 12 antibiotics if compared with isolates from otitis media and UTI (figure 2). The most resistance was seen to Aztreonam antibiotic with a percentage of 83%, while the most effective antibiotic was Vancomycin with a resistance percentage of (8%) (table 4).

(Diekema *et al.*, 2001) who shwed that more than 80% of coagulase-negative *staphylococcal* isolates were resistant to methicillin and semisynthetic penicillins. (Liu *et al.*, 2011) showed that Community-acquired MRSA (CA-

Table 4. Correlation between the type of infection and antibiotic resistance in *Staphylococcus* spp.

Infection \ Antibiotic		Amoxicillin+ Clavulanic acid 33%			Ceftriaxone 41%			Gentamycin 16%			Vancomycin 8%		
		s	i	r	s	i	r	s	i	r	s	i	r
UTI	NO	5	2	-	6	-	1	5		2	6	-	1
	%	71 %	29 %	0 %	86 %		14 %	71 %	-	29 %	86 %		14 %
Otitis media	NO	4	-	1	3	-	2	4	1	-	4	1	-
	%	80 %		20 %	60 %		40 %	80 %		0 %	80 %	20 %	0 %
Wound	NO	2	-	8	3	-	7	8		2	8	1	1
	%	20 %		80 %	30 %		70 %	80 %	-	20 %	80 %	10 %	10 %

Infection \ Antibiotic		Azactam 83%			Ciprobay 10%			Ceftazidime 52%			Azithromycin 53%		
		s	i	r	s	i	r	s	i	r	s	i	r
UTI	NO	-	-	7	7	-	-	3	-	4	5	-	2
	%			100 %	100 %		0 %	43 %		57 %	71 %		29 %
Otitis media	NO	-	2	3	4	1	-	3	-	2	1	-	4
	%		40 %	60 %	80 %	20 %	0 %	60 %		40 %	20 %		80 %
Wound	NO	-	1	9	4	-	6	4	-	6	5	-	5
	%		10 %	90 %	40 %		60 %	40 %		60 %	50 %		50 %

Infection \ Antibiotic		Ampicillin/cloxacillin 25%			Chloramphenicol 38%			Cephalothin 54%			Erythrocin 54%		
		s	i	r	s	i	r	s	i	r	s	i	r
UTI	NO	1	-	6	6	-	1	2	-	5	4	-	3
	%	14 %		86 %	86 %		14 %	29 %		71 %	57 %		43 %
Otitis media	NO	4	-	1	-	2	3	4	1	-	2	1	2
	%	80 %		20 %		40 %	60 %	80 %	20 %	0 %	40 %	20 %	40 %
Wound	NO	5	-	5	4	2	4	1	-	9	1	1	8
	%	50 %		50 %	40 %	20 %	40 %	10 %		90 %	10 %	10 %	80 %

Table 5. Correlation between the type of infection and antibiotic resistance in *Enterococcus* spp.

Antibiotic / Infection		Wound NO	Wound %	UTI NO	UTI %
Clarithromycin 69%	r	3	38 %	9	100 %
	i	-	-	-	
	s	5	62 %	-	
Erythrocin 88%	r	6	75 %	9	100 %
	i	-	-	-	-
	s	2	25 %	-	-
Nitrofurantoin 84%	r	5	63 %	3	34 %
	i	-	-	2	22 %
	s	3	37 %	4	44 %
Amoxyclav 71%	r	6	75 %	6	67 %
	i	-	-	2	22 %
	s	2	25 %	1	11 %
Chloramphenicol 0%	r	-	0 %	-	0 %
	i	-		-	-
	s	8	100 %	9	100 %
Azithromycin 0%	r	-	0 %	-	0 %
	i	1	13 %	-	-
	s	7	87 %	9	100 %
Vancomycin 89%	r	8	100 %	7	78 %
	i	-	-	2	22 %
	s	-	-	-	-
Ceftazidime 46%	r	1	13 %	7	78 %
	i	-	-	1	11 %
	s	7	87 %	1	11 %
Gentamycin 11%	r	-	0 %	2	22 %
	i	-	-	3	34 %
	s	8	100 %	4	44 %
Co-trimoxazole 57%	r	1	13 %	9	100 %
	i	-		-	-
	s	7	87 %	-	-
Ampicillin 65%	r	5	63 %	6	67 %
	i	-		-	-
	s	3	37 %	3	33 %
Cephalothin 72%	r	7	88 %	5	56 %
	i	-		-	-
	s	1	12 %	4	44 %
Ciprofloxacin 22%	r	-	0 %	4	44 %
	i	2	25 %	-	-
	s	6	75 %	5	56 %
Azactam 51%	r	1	13 %	8	89 %
	i	1	13 %	-	-
	s	6	74 %	1	11 %

MRSA) isolates often maintain susceptibility to tetracyclines (tetracycline, doxycycline, minocycline, tigecycline). The results by (Omoregie *et al.*, 2011) revealed that *Staphylococcus aureus* have great suseptibility to Amoxicillin (96.2%). Schwalbe *et al,*. (1987) showed that *Staphylococcus spp.* are resistance to Vancomycin.

Correlation between the type of infection and antibiotic resistance in *Enterococcus* spp.

Enterococcus was isolated from UTI and wound infections. The results revealed that isolates of UTI were more resistant than those isolated from wounds when tested against 14 antibiotics (figure 3) (table 5). (Karmarkar *et al.*, 2004) showed that 77.23% of enterococcal isolates were resistant to more than six drugs. (Herman and Gerding, 1991) studied serious enterococcal infections (*e.g.*, bacteraemia and endocarditis) and showed that such infections require treatment with a bactericidal combination of antibiotics that should include a penicillin (ampicillin or penicillin G) to which the isolates were susceptible and an aminoglycoside (gentamicin or streptomycin) to which they were resistant. (Courvalin, 2006) showed that more than 55% of enterococcal isolates in ICUs of more than 300 hospitals were vancomycin-resistant.

(Choudhury *et al.*, 2015) revealed that there were rare isolates that lack resistance aginst gentamicin and streptomycin, while high level of resistance were seen against amikacin and kanamycin. And Results obtained by (Sapkota *et al.*, 2007) revealed significant resistance by Enterococcus against erythromycin and tetracycline.

Correlation between the type of infection and antibiotic resistance in *Proteus* spp.

The isolates of *Proteus spp*. were obtained from wounds, UTI and otitis media. The most resistant isolates against 11 antibiotics were those isolated from wound infections (figure 4) The most resistance where against Ampicillin/cloxacillin (table 6). (Feglo *et al.*, 2010) showed that all proteus species were resistant to chloramphenicol, ampicillin and co-trimoxazole. However, 70 – 90 % of *P. mirabilis* and *P. vulgaris* isolates exhibited resistance to ampicillin, cotrimoxazole, tetracycline and chloramphenicol. (Newman *et al.*, 2006) revealed that *Proteus* spp. isolates exhibited high antimicrobial resistance against tetracycline

Table 6. Correlation between the type of infection and antibiotic resistance in *Proteus* spp.

Infection \ Antibiotic		Ceftriaxone 48%			Gentamycin 54%			Cephalothin 58%			Ceftazidime 41%		
		s	i	r	s	i	r	s	i	r	s	i	r
Otitis Media	NO	6	-	2	7	-	1	7	-	1	6	-	2
	%	75 %		25 %	87 %		13 %	87 %		13 %	75 %		25 %
UTI	NO	7	-	3	3	-	7	2	-	8	8	-	2
	%	70 %		30 %	30 %		70 %	20 %		80 %	80 %		20 %
Wound	NO	1	-	8	2	-	7	1	-	8	2	-	7
	%	11 %		89 %	22 %		78 %	11 %		89 %	22 %		78 %

Infection \ Antibiotic		Amoxyclav 61%			Ciprofloxacin 3%			Amikacin 42%			Chloramphenicol 58%		
		s	i	r	s	i	r	s	i	r	s	i	r
Otitis Media	NO	5	-	3	8	-	0	5	-	3	7	-	1
	%	62 %		38 %	100 %		0 %	62 %		38 %	87 %		13 %
UTI	NO	1	-	9	9	-	1	8	-	2	2	-	8
	%	10 %		90 %	90 %		10 %	80 %		20 %	20 %		80 %
Wound	NO	4	-	5	9	-	-	3	-	6	1	-	8
	%	44 %		56 %	100 %		0 %	33 %		67 %	11 %		89 %

Infection \ Antibiotic		Ampicillin/cloxacillin 66%			Cefixime 57%			Cefotaxime 50%		
		s	i	r	s	i	r	s	i	r
Otitis Media	NO	4	-	4	6	-	2	6	-	2
	%	50 %		50 %	75 %		25 %	75 %		25 %
UTI	NO	3	-	7	2	-	8	3	-	7
	%	30 %		70 %	20 %		80 %	30 %		70 %
Wound	NO	2	-	7	3	-	6	4	-	5
	%	22 %		78 %	33 %		67 %	44 %		56 %

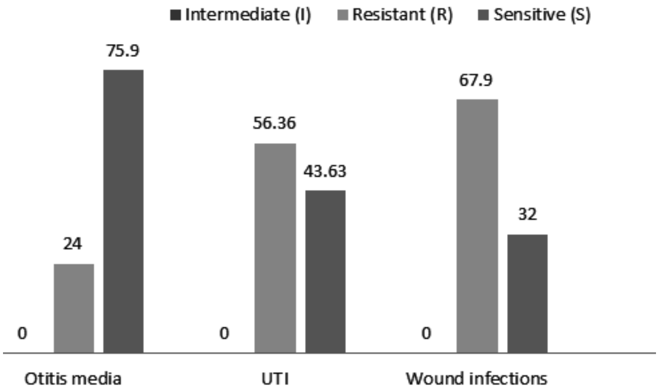

Fig. 4. The type of infection and antibiotic resistance in *Proteus* spp.

(85 %), chloramphenicol (82.5 %), co-trimoxazole (81 %) and ampicillin (77 %).

CONCLUSION

1. *E. coli* was the main cause of intestinal diseases.
2. *E. coli* was the most frequent bacteria casing UTI, *Proteus* spp. otitis media, and *Staphylococcus* spp. was the main cause of wound infections.
3. In males the infections with *Staphylococcus* spp. were more than in females, while infections with *E. coli*, *Proteus* spp. and *Enterococcus* spp. were more in females than in males.
4. In *E. coli* the resistance to antibiotics in UTI and intestinal disease was approximately the same.
5. In *Staphylococcus* spp. and *Proteus* spp. wounds infections exhibit more resistant than other types of infections.
6. In *Enterococcus* spp. UTI infections exhibit more resistant than wounds infections.

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