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Sensitivity and Resistance of Main *Staphylococci* Isolated of Urine Samples Cause UTIs at the National Hospital in Qamishly

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□ ABSTRACT □

Antimicrobial resistance is a big problem that needs a healthy and serious care.

The current study researches 39 *staphylococcus* isolated from urine of patients and outpatients and the antimicrobial resistance. The research carried out at the National Hospital in Qamishly during the period 2008 - 2009. Results showed that the rate of urinary tract infections was more in females (94.87%) than in males (5.12%) and more in adults (87.17%) than children (12.82%). We found that all the strains of isolated bacteria showed high susceptibility against Imipenem (94.82%), Cefazoline (83.84%), Ceftriaxone (69.23%), Cefaclor (69.2%), Amikacine (64.1%), and Gentamycine (61.5%). In addition, most isolated bacteria showed intermediate Sensitivity of Ciprofloxacin (53.8%), Levofloxacin (51.2%), and rate of sensitivity against Tobramicine (38.4%), Amoxicilline clavolanic acid (25.6%), Pefloxacine (25.6%), Vancomycine (25.6%). At the same time, most of the isolated bacteria showed resistance of Ceftazidime, Cefotaxime, Cefuroxim sodium, Ofloxacin, Cefoxitin, Sulphamethoxasole trimethoprim, Tetracyclines, Erythromycin, Penicillin, Ampicillin, Oxacillin, Amoxicilline.

Key Words: Bacteria, Resistance, Antibiotics, Ear, National Hospital in Qamishly.

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الحساسية والمقاومة لأهم العنقوديات المعزولة من عينات البول المسببة للإنتانات البولية في المستشفى الوطني في القامشلي

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□ ملخّص □

تعدّ إنتانات المسالك البولية لدى الرجال والنساء الناتجة عن جراثيم العنقوديات أحد أهم الإنتانات، وتبرز مقاومة الجراثيم للصادات الحيوية كمشكلة كبيرة ذات اهتمام صحي كبير، وتكمن خطورتها في ازدياد قدرة الجراثيم على تطوير وتغيير مقاومتها للصادات الحيوية.

أُجريت هذه الدراسة على 39 عزلة من العنقوديات المعزولة من البول ومقاومتها للصادات الحيوية لدى المرضى المراجعين والمقيمين في المستشفى الوطني بالقامشلي في الفترة بين 2008 – 2009، وكانت النسبة المئوية للإنتانات في الإناث 94.87% أعلى مما هي عليه لدى الذكور 5.12%، والنسبة المئوية للإنتانات في البالغين 87.17% أعلى مما هي لدى الأطفال 12.82%، وأبدت هذه الجراثيم حساسية عالية تجاه كل من الإيميبينيم 94.82%، والسيفازولين 83.84%، والسيفترياكسون 69.23%، والسيفاكلور 69.2%، والأميكاسين 64.1%، والجنتاميسين 61.5%، وحساسية متوسطة للسيبروفلوكساسين 53.8%، والليفوفلوكساسين 51.2%، وحساسية بنسبة 38.4% للتوبراميسين، والأموكسيسيلين كلافولانيك أسيد 25.6%، والبيفلوكساسين 25.6%، والفانكوميسين 25.6%، ومقاومة للصادات الحيوية الآتية: السيفتازيديم، والسيفوتاكسيم، والسيفوروكسيم صوديوم، والأوفلوكساسين والسيفوكسيتين، والسلفاميثوكسازول تريميثوبريم، والتتراسيكلين، والإرثروميسين، والبنسيلين، والأمبيسيلين، والأوكساسيلين، والأموكسيسيلين.

الكلمات المفتاحية: الجراثيم، المقاومة، الصادات الحيوية، البول، المستشفى الوطني بالقامشلي.

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Introductions:

Staphylococci are Gram-positive cocci in the family Staphylococcaceae. These organisms are part of the normal human flora; 25 – 50% of healthy persons and may be persistently or transiently colonized, nonmotile, aerobic, and facultatively anaerobic (Fauci *et al*. 2008).

The configuration of the cocci helps to distinguish *staphylococci* from *streptococci*, which are slightly oblong cells that usually grow in chains (because they divide in one plane only). The catalase test is important in distinguishing *streptococci* (catalase-negative) from *staphylococci* catalas positive, which are vigorous catalase-producers (Todar, 2004 www.textbookofbacteriology.net). *Staphylococcus aureus* forms a fairly large yellow colony on rich medium. *S. epidermidis* has a relatively small white colony. *S. aureus* is often hemolytic on blood agar, but *S. epidermidis* is non hemolytic. *Staphylococci* are facultative anaerobes that grow by aerobic respiration or by fermentation that yields principally lactic acid.

The bacteria are catalase - positive and Oxidase-negative. *S. aureus* can grow at a temperature range of 15 to 45 degrees and at NaCl concentrations as high as 15 percent or more. Nearly all strains pathogenic of *S. aureus* produce the enzyme coagulase: nearly all strains of *S. epidermidis* lack this enzyme. *S. aureus* should always be considered as a potential pathogen; most strains of *S. epidermidis* are nonpathogenic and may even play a protective role in their host as normal flora. *Staphylococcus epidermidis* may be a pathogen in the hospital environment (Gladwin *et al*. 2000; Jawetz & Levinson, 1998).

Over 30 different types of *Staphylococci* can infect humans, but most infections are caused by *Staphylococcus aureus*. *Staphylococci* can be found normally in the nasal membranes and on the skin (and less commonly in other locations) of 20-30% of healthy adults. In the majority of cases, the bacteria do not cause disease. However, damage to the skin or other injury may allow the bacteria to overcome the natural protective mechanisms of the body, leading to infection (Gladwin *et al*. 2000; Fauci *et al*. 2008). *Staphylococci* can cause a multitude of diseases as a result of infection of various tissues of the body such as skin infection, throat, Septicaemia, Respiratory infections, Bacteremia, Sepsis, and Infective Endocarditis, Ear infections, and Urinary Tract Infections (Gladwin *et al*. 2000; Fauci *et al*. 2008), *Staphylococci* bacteria can cause illness not only directly by infection (such as in the skin), but indirectly by producing toxins, responsible for food poisoning and toxic shock syndrome. Staph-related illness can range from mild and requiring no treatment to severe and potentially fatal (Todar, 2004 www.textbookofbacteriology.net), and *Staphylococci* are one kind the main bacteria that cause the infections in the Hospital, and it causes acute infection of the urinary tract fall into two general anatomic categories: lower tract infection (urethritis and cystitis) and upper tract infection (acute pyelonephritis, prostatitis, and intrarenal and perinephric abscesses) (Brenner 2008; Fauci *et al*. 2008).

Many microorganisms can infect the urinary tract, but by far the most common agents are the gram-negative bacilli, like (*E. coli* 75-85%) while Gram-positive cocci play a lesser role in UTIs. However, *Staphylococci* cause about 10–15% of acute symptomatic UTIs in young female patients.(Wise 1993; Fauci *et al*. 2008).

The antimicrobial resistance is a big problem that needs a healthy and serious care, and its danger lies in the ability of bacteria in developing and changing resistance against the antibiotics, and showing resistant strains in life increases day by day, generally the bacteria resist the antibiotics by many several agents, some of these agents caused by Plasmids (extra chromosomal DNA), and other caused by non genetic agents like the

proteins in the capsule structure, uncontrollable Antibiotics use in the medical unit care and hospitals (Wickens & Wade, 2005).

Diseases and disease agents that were once thought to be controlled by antibiotics are returning in new leagues resistant to these therapies, and MRSA is a common cause of infection among hospitalized patients (Levy & Marshall, 2004; kekre, 2010). It is clear that bacteria will continue to develop resistance to currently available antibacterial drugs by either new mutations or the exchange of genetic information, that is, putting old resistance genes into new hosts (Tenover, 2006).

In many healthcare facilities around the world, bacterial pathogens that express multiple resistance mechanisms are becoming the norm, complicating treatment and increasing both human morbidity and financial costs day by day, therefore, the prudent use of antibacterial drugs, using the appropriate drug at the appropriate dosage and for the appropriate duration, is one of the important means of reducing the selective pressure that helps resistant organisms emerge (Storz & Hengge**,** 2000; Tenover 2006), and because of increasing of infectious disease in older persons, adults and children in many area of the world and those infections associated with high morbidity, mortality and susceptibility (Yoshikawa, 2002). Therefore many studies and researches still study antimicrobial resistance and sensitivity to give important reports for any medical range. In Syria, one of the studies in Alasad University Hospital in Lattakia showed the percent of the *Staphylococcus aureus* which causes UTIs in was 8.2%, and sensitivity was high to gentamycine, amikacine, while it is resistant to ampicilline and amoxicilline clavolanic acid (balash *et al*. 2006). Outside Syria, some studies, showed the increase of the prevalence of UTIs which is caused by *Staphylococci* in women in nine American areas (Gupta *et al*. 2001), and the percent of infections which is caused by *Staphylococcus aureus* was changed in one of the hospitals in Taiwan from 5.2 % to 12.1 %, and the percent of antimicrobial resistance from 20% in eighteenth to 60.2% in nineteenth (Ren Hsueh *et al*. 2002). *Staphylococcus aureus*, *S. epidermidis*, *S. saprophyticus* were the most distributive in UTIs in Pakistan (Gul *et al*. 2004), and the prevalence of UTIs which is caused by *Staphylococci* in India was 23%, and the prevalence of UTIs was high in females more than males (Jha & Bapat, 2005). In Japan the prevalence of UTIs which is caused by *Staphylococci* increased from 1.9% in 1987 to 6.6% in 2002, and the sensitivity was changed within these years (Shigemura *et al*. 2005), in Iran was shown that the coagulase negative *staphylococci* in urine was 10.1%, *Staphylococcus aureus* 3.22%, and 66.6% of it was resistant to vancomycine (Ashteiani *et al*. 2007). In several hospitals in India it was shown that prevalence of UTIs which is caused by *Staphylococci* was 23%, and the prevalence of UTIs was higher in females than males, and this *Staphylococci* was resistant to penicillins (Hasan *et al*. 2007). In some hospitals in Nigeria *Staphylococcus aureus* (22.8%) was the most between the *Staphylococci* which causes UTIs and these *Staphylococci* were sensitive to nitrofurantein, ofloxacin, and were resistant to tetracycline, nalidixic acid (Akortha & Ibadine, 2008), while in Turkey the percent of pathogen *staphylococci* was 18%, and the MRSA 90% (Inceck *et al*. 2009). There are variety and big changes in patterns and percents of antimicrobial resistance in UTIs especially in the children (Chakupurakal *et al*. 2010), and the percent of *Staphylococcus aureus* was isolated from urine which cause UTIs was 20.5% in India (Manikandan *et al*. 2011). In Jordan the percent of *Staphylococcus saprophyticus* which cause UTIs arrived to 4.8 % in a rural area (Nimri & Batchoun, 2011).

Aims of the study:

1. Study the main extension of *staphylococcus* sp. That causes urinary tract infections and at The National Hospital in Qamishly.

2. Study of sensitivity and resistance of pathogenic *staphylococcus sp.* causing UTIs and antibiotics resistance at The National Hospital in Qamishly.

3. To get practical, important results which help the clinical doctors in the description of the occasion antibiotics for patients in this area.

1. Materials

1.1 Culture Media: Nutrient Agar (Hi media India), Manitol salt agar (Criterion England), Mueller Hinton Agar (Biomark India).

1.2. Antibiotics

Antibiotic	code	Antibiotic	Code
Amoxicilline	Ax 25 mcg	Amoxicillin\ clavolanic acid	AMC30mcg
Ampicillin	AM10mcg	Cefaclor	CEC30mcg
Cefoxitin	FOX30mcg	Ceftazidime	CAZ30mcg
Cefuroxim Sodium	CXM30mcg	Cefazoline	CZ 30 mcg
Ceftriaxone	CRO 30	Imipenem	IPM 10mcg
Ciprofloxacin	CIP 5 mcg	Cefotaxime	CX30mcg
Amikacine	AK30mcg	Sulphamethoxazol	SXT25mcg
Ofloxacin	OFX5mcg	Tobramicine	Tob 10mcg
Gentamycine	GM 10mcg	Levofloxacin	LEV 5 mcg
Pefloxacine	PE5mcg	Penicillin G	P 10mcg
Oxacillin	OX 1mcg	Erythromycin	E 15 mcg
Vancomycine	VA 30mcg	Tetracycline	TE 30mcg

2. Methods

2.1 **Samples**

A total of 75 urine samples were collected. We isolated 39 of *staphylococcus*; all samples were taken before administration of antibiotics from the out patients and inpatients in our hospital during the study period (01 / 08 / 2008 to 31/ 10/ 2009). The mid-stream urine specimens were obtained by clean – catch method. The samples were collected in sterile containers and cultured within half an hour of collection.

2.2 **Microscopic diagnosis**

Urine specimen was detected directly by the microscope after the centrifugation, and account for the white cell in the urine, so that the existence is more than 10^4/ml in urinal precipitations, and the existence of bacteria in the microscopic field generally indicate infection (Henry, 2001).

2.3. **Specimen culture**

From a microbiological perspective, urinary tract infection (UTI) exists when pathogenic microorganisms are detected in the urine. In most instances, growth of $\geq 10^5$ CFU/ml or more from a properly collected midstream "clean-catch" urine sample indicates infection. However, significant bacteriuria is lacking in some cases of true UTI (Fauci *et al*. 2008).

The samples were plated out on Nutrient Agar, and incubated aerobically overnight at 37º and after the growth were plated out on Manitol salt agar, at the same time we performed catalase test, and coagula test to identify the pathogenic *Staphylococcus* (Henry, 2001; De lamaz *et al*. 1997)

2.4 **Statistical analysis**

The results were analyzed statistically using various methods, including Spearman correlation, and One-Way Anova in level 0.05. All statistical analyses were conducted using version 17 of the SPSS program.

2.5 **Antibiotic susceptibility Testing**

Antimicrobial sensitivity testing of all isolates was performed on diagnostic sensitivity test plates by the Kirby Bauer method, following the definition of the National Committee of Clinical Laboratory Standards (NCCLS) (Henry, 2001).

Bacterial Inoculum was prepared by suspending the freshly – grown bacteria in 25 ml sterile nutrient broth. A sterile cotton swab was used to streak the surface of Mueller Hinton agar then incubated at 37º. Filter paper discs containing designated amounts of the antimicrobial drugs obtained from commercial supply firms (Biomark India, Gouhan labs Turkey) were used.

After measuring the diameters of the clear zones around the antimicrobial discs and following the procedures of the National Committee for Clinical Laboratory Standards, the strains were categorized as sensitive or resistant to the drug (Fauci *et al*. 2008; Henry, 2001).

3. Results & Discussion

3.1 Extension of the percentage of infections between the two sexes

The percentage of the UTIs was more common in females (94.87%) as compared to males (5.12%). The differences between the sexes were subjected to Spearman correlation statistical test and it showed that there was significant difference of the percentage between sexes. This extension is shown in **Table 1.** The results corresponds to results obtained in similar studies conducted in Nigeria like (Akortha & Ibadine, 2008), that the percentage of infections in females 65.8%, and males 34.2%, and study of (Adedeji & Abdulkadir, 2009), which the percentage of infections in females was 52%, and males 32%, and agree with similar studies in India like (Hasan *et al*. 2007), and (Nandy *et al*. 2007). Most infections are caused by retrograde ascent of bacteria from the faecal flora via the urethra to the bladder and kidney especially in females who have a shorter and wider urethra and is more readily transferred by microorganisms. The structure of the females urethra and vagina makes it susceptible to trauma during sexual intercourse as well as bacteria been massaged up the urethra and into the bladder during pregnancy and or childbirth (Brenner 2008; Fauci *et al*. 2008).

Table 1. Extension of the percentage of infections between the sexes.

Sex	Number	Percent%
Male	2	5.12
Female	37	94.87

3.2 Extension of the percentage of infections between the ages

The percentage of the UTIs was more common in adults (87.17%) as compared to children (12.82%). The differences between the ages were subjected to Spearman correlation statistical test and it showed that there is no significant difference of the percentage between ages. This distribution is shown in **Table 2.**

Comparing the results of our study with those in a similar study, we find that they agree with many studies like (Jha & Bapat, 2005) in India in which the percentages of UTIs are higher in adult females (21-30 year), and adult males (31-40 year).These results suggest that this is the sexually active and also the child-bearing age group.

Table 2. Extension of the percentage of infections between the Ages.

Ages	Number	Percent%
Children 1-15	5	12.82
Adults above 15	34	87.17

3.3 The percentage of sensitivity and resistance of isolated *staphylococcus sp.* to used antibiotics in this study

Antimicrobial susceptibility results are summarized in **(Table 3, Figure. 1).**

The high sensitivity pattern of isolated *staphylococcus* in this study is 94.82, 83.84, 69.23, 69.2, 64.1, and 61.5%, to Imipenem, Cefazoline, Ceftriaxone, Cefaclor, Amikacine, and Gentamycine, respectively, and the intermediate sensitivity is 53.8, 51.2, and 38.4%, to Ciprofloxacin, Levofloxacin and Tobramicine, respectively, and is 25.6% to AMC acid, Pefloxacine, and Vancomycine, while *staphylococci* isolates are resistant to Ceftazidime, Cefotaxime, Cefuroxim sodium, ofloxacine, Cefoxitin, Sulphamethoxazol trimethoprime, Tetracycline, Erythromycin, penicillin, Ampicillin, Oxacillin, and Amoxicilline. The differences between the sensitivities were subjected to One Way Anova statistical test and it showed that there is a significant difference of the percentage between these results (<0.05).

Table 3. The percentage of sensitivity and resistance of isolated *staphylococcus* sp. to used antibiotics.

Antibiotics mcg	Number	(S) %	(I) %	(R) %
Amoxicillin\ clavolanic acid AMC 30	39		25.64	
Levofloxacin LEV 5	39		51.2	
Ciprofloxacin CIP 5	39		53.8	
Pefloxacin PE 5	39		25.64	
Ofloxacin OFX 5	39			7.69
Gentamycine GM 10	39	61.5		
Tobramycine TOB 10	39		38.46	
Amikacine AK 30	39	64.1		
Vancomycine VA 30	39		25.64	
Cefuroxim Sodium CXM 30	39			12.82
Ceftriaxone CRO 30	39	69.23		
Cefotaxime CX 30	39			18.1
Ceftazidime CAZ 30	39			10.25
Cefoxitin FOX 30	39			2.56
Cefaclor CEC 30	39	69.2		
Cefazoline CZ 30	39	83.84		
Imipenem IPM 10	39	94.82		
Sulphamethoxazol trimothoprim SXT 25	39			5.12
Penicilline P G 10	39			0
Amoxycillin AX 25	39			7.69
Ampicillin AM 10	39			7.69
Oxacilline OX 10	39			2.56
Tetracycline TE 30	39			2.56
Erthromycin E 15	39			2.56

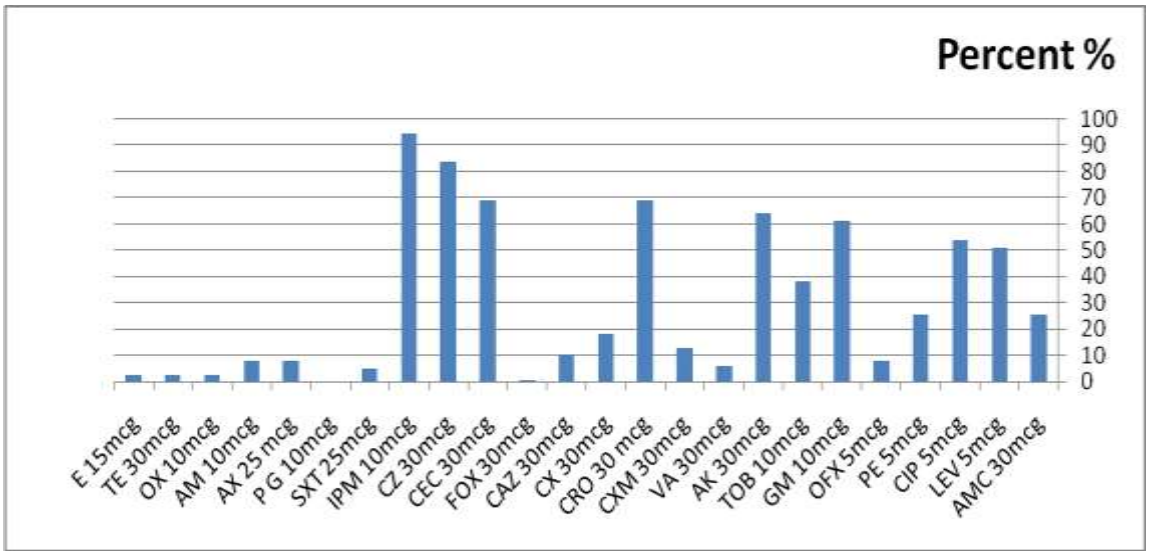

Figure. 1. The percentage of sensitivity and resistance of isolated *Staphylococcus* sp. to used antibiotics.

Sensitivity patterns of *Staphylococcus* to antibiotics recorded by other studies show similarity except in few cases. In the study carried out by(Gul *et al*. 2004) in Pakistan on antimicrobial susceptibility patterns, 46.1, 76.9, 30.7, 76.9, and 46.1%, were reported as sensitive to Ciprofloxacin, Ofloxacine, SXT, Gentamycine, and Tobramicine, respectively, while resistance was recorded against penicillin, and tetracycline. Also (Vasquez & Hand, 2004) in the USA, showed the isolated *staphylococci* as being 88.9% to Imipenem, 90% to Cefazoline, Cefotaxime, ciprofloxacin and Levofloxacin, 100% to gentamycin and Vancomycine, and resistant to Ampicillin **(Table 4, figure 2)**. The results in one of (Akortha & Ibadin, 2008) studies in Nigeria were 50.2% sensitive to Gentamycine, but were 83% to AMC acid, and 75.9% to Ofloxacine, and resistance to tetracycline 19.1%. Also in contrast to (Bhargavi *et al*. 2010) study, reported *staphylococci* as 55.6% sensitive to Gentamycine, and 40% to Amikacine and Ceftazidime, and 36.4% to Ciprofloxacin. In the last contrast to (Oladeinde *et al*. 2011) study in Nigeria on antimicrobial susceptibility patterns, 72.7, 65.7, 54.5, and 46.9% were reported as sensitive to Ciprofloxacin, Pefloxacine, Ofloxacine, and Gentamycine, respectively, while resistance was recorded against, AMC acid (9.1%), SXT (4.5%), and Amoxicilline (1.5%) **(Table 4, figure 2)**. These differences in sensitivity patterns of *Staphylococci* could be attributed to environmental factors such as the misuse and abuse of antibiotics among the general population, which has favored the emergence of resistance strains just as it could be the case in other organisms in any particular region or community.

Table 4. The comparison between results of sensitivity in our study and studies (Vasquez & Hand 2004, Oladeinde *et al.* 2011).

Antibiotics, mcg	Percentage (%) of Sensitivity & resistance of *Staphylococci*		
	Our study	Vasquez & Hand	Oladeinde *et al.*
AMC 30	25.64	80	9.1
LEV 5	51.2	90	-
CIP 5	53.8	90	72.7
PE 5	25.64	-	65.7
OFX 5	7.69	-	54.5
GM 10	61.5	100	46.9
TOB 10	38.46	-	-
AK 30	64.1	-	-
VA 30	25.64	100	-
CXM 30	12.82	-	-
CRO 30	69.23	-	-
CX 30	18.1	90	-
CAZ 30	10.25	-	-
FOX 30	2.56	-	-
CEC 30	69.2	-	-
CZ 30	83.84	90	-
IPM 10	94.82	88.9	-
SXT 25	5.12	100	4.5
P G 10	0	-	-
AX 25	7.69	-	1.5
AM 10	7.69	5.5	-
OX 10	2.56	90	-
TE 30	2.56	90	-
E 15	2.56	-	-

(-) it means that antibiotics not experiment in their studies

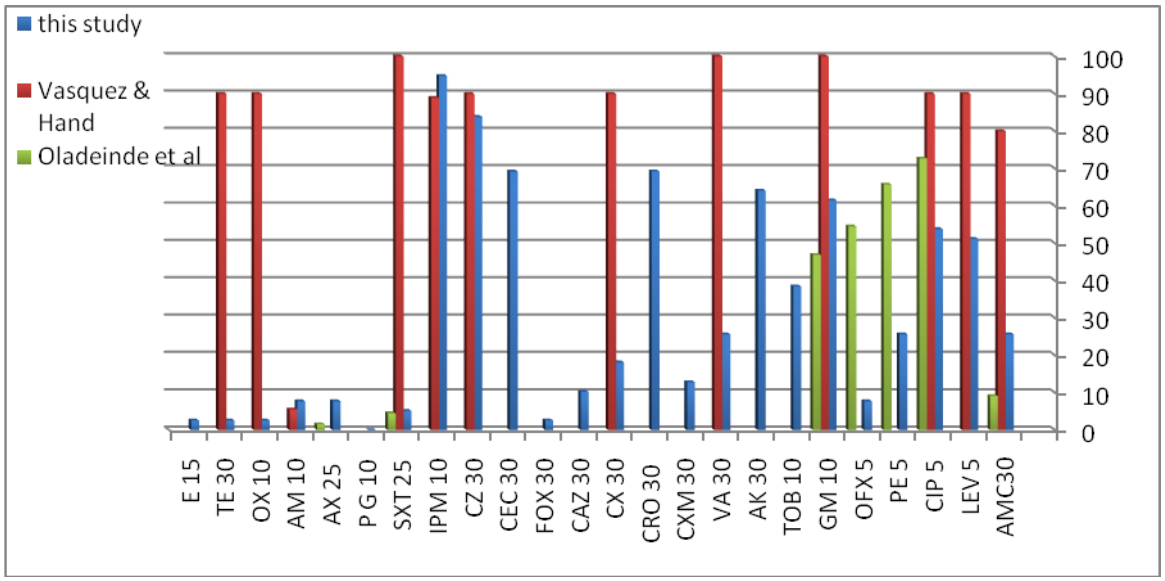

Figure 2. The comparison between results of sensitivity in this study and Vasquez & Hand 2004, Oladeinde *et al.* 2011 studies.

Recommendations:

1. For many reasons, the antimicrobial resistance of *staphylococci* which cause UTIs to antibiotics causes a very serious problem, because of its resistance and development among the bacterial strains. Doctors must carry bacterial culture and sensitivity to antibiotics to know the species of bacteria which cause UTIs.

2. Showing forms of strong bacteria because of the environmental factors such as the misuse and abuse of antibiotics among the general population.

3. For clinical doctors they should be careful when they describe poisonous antibiotics especially for children.

4. Many studies and researches must be carried in all areas to observe the extension of bacteria, and antibiotics which affect.

5. Reply to these studies in the same area to observe development, and increasing of resistance against antibiotics.

6. Work to help for healthy culture and awareness especially about antibiotics uses.

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