STUDY OF OPPORTUNISTIC BACTERIA ISOLATED FROM NOSOCOMIAL INFECTION

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ABSTRACT

During this study 46 samples were collected from Shahed Kalid Koya hospital obtained from (Kitchen room, intensive care unit, operation theater, and labor ward). a microbiological surveillance was done, that contribute to the circulation of germs around the patient from these units were isolated (five isolates *Micrococcus varians*, seven isolates *Staph epidermis*, nine isolates *Escherichia coli, ten* isolates *Candida albicans*, three isolates *Lactobacillus sp.*, five isolates *Pseudomonas aerogenosa*, one isolate *Listeria ivanovii*, one isolate *Citrobacter freundii*, nine isolates *Bacillus subtilius*, one isolate *Enterobacter pnemonia*, six isolates *Corynebacteria sp.*). All these isolates were identified according to the cultural characteristics, morphological, and biochemical examination. The antibiotic susceptibility test were conducted to fifteen antimicrobials agents including (gentamycin (CN), chloramphenicol(Ch), ciprofloxacin(Cip), cefotaximsodium(CTX), amikacin(Amk), pipracillin(Pip), rifampicin(RD), doxycillin (DO), trimethoprim (Tri), pyopein (py), cloxacillin (CX), ampicillin (Amp), erythromycin (E), tobramycin (Tob) and Kiflex (KF). Antibiotic susceptibility which appears the highest resistant rate observed for (RD, Tri, Amp and KF) 100% respectively, CN90% and lower resistance rates were observed for (Cip and Pip) 0%respectivelyand DO 28.57%.

Key words: Opportunistic Bacteria; multidrug-resistant bacteria.

INTRODUCTION

Hospital-acquired infections are a considerable problem for health services in all countries, with serious effects on the survival of high-risk patients, such as burn patients. In a burns centre, primary bloodstream infections, pneumonia, and infection of burn sites are very dangerous complications that can compromise the patient's survival and the outcome of reconstructive treatment. We stress the importance of emerging pathologies involving opportunistic micro-organisms. .

In many patients we have detected infections caused by *Pseudomonas* spp. The isolation of *Pseudomonas* spp., *Bacillus cereus*, *Achromobacter*, *Acinetobacter*, and *Proteus* spp. in some patients in the Palermo Burns Centre was the warning signal that prompted an investigation for the presence of opportunistic bacteria in the environment (Torregrossa *et al*., 2000).

Nosocomial infections are infections that are a result of treatment in a hospital or a healthcare service unit. Infections are considered nosocomial if they first appear 48 hours or more after hospital admission or within 30 days after discharge. Nosocomial infections are commonly transmitted when hospital officials become complacent and personnel do not practice correct hygiene regularly. Also, increased use of outpatient treatment means that people who are hospitalized are more ill and have more weakened immune systems than may have been true in the past. Moreover, some medical procedures bypass the body's natural protective barriers. Since medical staff moves from patient to patient, the staff themselves serves as a means for spreading pathogens.(Ricks, 2007).

Almost any microbe can cause a hospital-acquired infection, though protozoal infections are rare. The pattern of hospital infection has changed over the years, reflecting advances in medicine and the development of antimicrobial agents. In the pre-antibiotic era the majority of infections were caused by Gram-positive organisms, particularly *Streptococcus pyogenes* and *Staphylococcus aureus.* With the advent of penicillin and other antibiotics active against staphylococci, Gram-negative organisms such as *Escherichia coli* and *Pseudomonas aeruginosa* emerged as important pathogens. More recently, the development of more potent and broad spectrum antimicrobials and the increase in invasive medical techniques has been accompanied by an increase in the incidence of: antibiotic-resistant Gram-positive organisms such as coagulase-negative staphylococci, enterococci and methicillin-resistant *Staph. aureus* (MRSA); multidrug-resistant Gram-negative organisms including those producing expanded spectrum beta-lactamases (ESBLs), Many of these organisms are considered as 'opportunists'-microbes that are unable to cause

disease in healthy people with intact defense mechanisms, but can cause infection in compromised patients or when introduced during the course of invasive procedures. Currently *E. coli* accounts overall for more hospital infections than any other single species, but staphylococci are a close second and after that *Candida* (Cedric *et al*., 2007).

The aims of this study were isolation and identification of opportunistic microorganisms that cause nosocomial infection collected from different sources of operation, kitchen room, intensive care unite, and labour ward by cultural, morphological and biochemical tests including API 20E test, then Study the resistance of opportunistic microorganisms to different antimicrobials agents.

MATERIALS AND METHODS

Forty six samples were collected from operation unit, kitchen room, labour unit and intensive care ward from Koya shahed kaled hospital. **Environmental sampling:-** The survey of the level of microbiological environmental pollution was carried out of (Ground, wall, articles, operation bed, disinfectants (dettol and habetten), surgical gloves , white coat, light, pressure device, dining table, pots, and table of cafeteria, refrigerators, large spoon, plate, glasses, baby balance, and baby doplex . **Staff sampling: -** monitoring was carried out by a culture test of nasal and skin swabs to test for pathogens. **Isolation and Identification of Bacterial strains**: Forty six samples were obtained from unites of Shahed Kalid Koya hospital, used Brain-Heart infusion broth (Rashmi Diagnostics, India) and transferred to the laboratory, and 11 different types of microorganisms were identified by colony identification on different types of agar like (blood, MacConky, Citremide, Eosin methylene blue EMB, and Potato dextrose agar PDA) agar.

The biochemical tests of suspected bacteria that were detected by IMVC (Indole, methyl red (MR), vogesproskauer (VP) and Simon's citrate agar), triple sugar iron agar (TSI agar) (Mast diagnostic U.K.), urease, gelatinase, oxidase and catalase test H2S production (Atlas *et al*, 1995).

Antibiotic susceptibility testing: Antibiotic susceptibility test was conducted for these isolates, Muller Hinton agar (Difco U.S.A.) was used as growth medium, after sterilization and cooling at 45˚C, the plates were inoculated by streaking method with microorganisms then incubated at 37˚C for 24 hours. The results were recorded next day (Crump, J. *et al*., 2004). The isolates were tested for antimicrobial susceptibility by disc diffusion technique according to NCCLS guidelines (National committee for CLS, 2000). The following antibiotic discs (drug concentration in µg) were used: Amikacin 30µg, Ampicillin30µg, Arethromycin 10µg, Cefotaxime sodium 30µg, Kiflex 30 µg, Chloramphenicol 30µg, Gentamicin10µg, Rifampin 5µg, Piperacillin 100 µg, Tobramycin 10 µg, , Cloxacillin, Doxycillin 15µg, ciprofloxacin 30 µg and Trimethoprim10µg.

RESULTS AND DISCUSSION

Isolation and Identification of microorganisms: The characteristics of isolates were studied, through culturing them on differential medium, according to smear preparation by Gram stain the bacterial cells are some of them Gram negative, rods, short, motile, non spore forming and others gram positive bacilli spore forming and some of them appear as cluster shape irregular arrangement, some of microorganisms appear Fungi (*Candida albicans*) observations (Cruicksahnk *et al*., 1975; and Warren and Jawtez., 2000).

Specimen collection: forty six samples collected from operation department, Kitchen room, intensive care ward, labour ward from different sources, environment, articles and staff members that show in table (1, 2, 3, 4).

1-Escherichia coli: six isolates were obtained from no. (4,9,10,11,12,15,16,17,20) (Table of cafeteria, dining table, refrigerators no. 1and no. 2, cooker, plate, glass, nasal swab) respectively from kitchen room as show in table (2), which indicate that these isolates were ***E. coli,*** through culturing them on differential medium, such as MacConkey agar. In this case, the isolates appear smooth and circular, and pink in color by fermenting lactose, on EMB media the isolates are highly pigmented and have small smooth metallic sheen colonies).

2-Pseudomonas aeruginos: five isolates was obtained from No. (6, 7, 8, 9) (Pot,table, ground, wall) respectively from kitchen room as show in table (2), and (No. 3) in table (3) from intensive care unit. The colonies of ***P. aeruginosa*** isolates were studied using nutrient agar and MacConkey agar. They are small in size, smooth in appearance with flat edge and an elevated appearance, most of these isolates

produce pyocyanin (blue green pigment), and ***P. aeruginosa*** does not ferment lactose and the bacterial colonies were able to grow at 41C° but not at 4C° these criteria are used for the identification of ***P. aeruginosa*** from other species.The bacterial cells from smear preparation are Gram negative, rod-shaped, and occur as single in pairs or in short chains, and presumptively are regards ***P. aeruginosa*** (Todar, 2004).

3-Citrobacter freundii: Citrobacter is a genus of Gram-negative coliform bacteria in the Enterobacteriaceae family, this isolate appear smooth and circular, and pink in color by fermenting lactose on MacConkey agar, one strain isolated from spoon (No. 14) that obtained from kitchen room.

4-Enterobacter pneumonia one isolate was obtained from Sacker device on MacConkey agar appear small pink color colony, on triple sugar iron agar ferment both glucose and lactose and Gas is produced.

***5-Staphylococcus epidermidis*:** Staphylococcus epidermidis is Gram positive cocci in clusters, facultative anaerobe, Non motile not spore forming do not have capsule. **Biochemical tests:** Ferment glucose and lactose, positive to (vogusproskaure, catalase), Negative to (indole, H2S, Coagulase , DNase, oxidase-, bile esculin) (Salyers *et al.*, 2002), seven isolates were obtained from Sacker device, White coat, gloves, skin swab, and nasal swab.

6-***Bacillus subtilis:*** is a Gram-positive rod-shaped, and has the ability to form a tough, protective, allowing the organism to tolerate extreme environmental conditions aerobic or facultative anaerobic (Todar, 2011). Thirteen isolates of ***Bacillus subtilis*** were obtained from Shahed kalid hospital five of these isolates obtained from kitchen room no. (4, 5, 6, 11, 18) for (table of cafeteria, bowl, pot, refrigerator, and skin swab) respectively, operation department isolate no. 4 obtained from bed, from intensive care unit isolates no. (1, 2, 3) obtained from (wall, ground, table) respectively and labour ward isolates no. (1, 2, 6, 8) obtained from (ground, wall, balance baby dopplex) that demonstrate in table (1-2-3-4).

7-Listeria ivanovii: Gram-positive rods, non spore forming, aerobic, motile bacteria and the bacterial colonies were able to grow at 4C.° listeria was classified in the family Corynebacteriaceae (Ermolaeva, *et al.*, 2003), one isolate was obtained from no. (7) Table of kitchen room.

8-Micrococcus varian: on blood agar, the isolates appear smooth and circular, Since micrococcus is pigmented bacteria, it produces either yellow or reddish colonies when unstained this strain yellow in color and not production haemolysis on sheep blood agar, on EMB agar have smooth pink color colony, (Smith, et *al.* 1999) obtained ten isolates from kitchen room no. (1,8,9,10,13,14,15,16,18,19) (nasal swab from someone of the staff, ground, wall, dining table, plate, spoon, cooker, plate, skin swab and nasal swab) respectively appear in table (1, 2,3 and 4).

9-Lactobacillus spp.: Lactobacilli are none sporulating, Gram-positive bacilli classified in the large family Lactobacillaceae Long, narrow rod occurring in long chains, on blood agar tiny colonies often alpha-hemolytic, Catalase-negative. Lactobacilli, commonly contaminating commensals. (Dicks, 2000) *Lactobacillus spp.* obtained from Oxygen device and glass.

*10-**Corynebacteria species:*** Gram-positive non-spore forming bacilli with non-parallel sides and wider ends resulting in club shaped forms (coryneform) Arranged as single cells, pairs, V, L, and Y (diphtheroid) Non-β-hemolytic on sheep blood agar and wet smooth colony gray in color and Grow well on sheep blood but not enteric agar (MacConkey), Positive for catalase test (Yoshihito, *et al.* 2005). Some isolates which indicate in table (1) obtained, from bed in table (2) from table of cafeteria and bowel, in table (4) from ground.

11-*Candida albicans:* Isolates obtained from operation department no. (3, 9, 10, 11) for (articles, white coat, light, pressure device) respectively, from intensive care isolate no.(1, 2 and 4) for (wall, ground, and bed) respectively and from Labour ward isolate no. (7) appear in table (1,2, 3 and 4) . on blood agar, the isolates appear white smooth colony. Candida is gram positive, and it grows overnight on most bacterial and fungal media (Di Salvo, 2010).

Table (1) microorganisms that isolated from Operation department

No. of samples	Samples	Results
1	Ground	*N.G.*
2	Wall	*N.G.*
3	Operation articles	*Micrococcus varian*
4	Bed	*Corynebacteria spp.*
5	Disinfectant	
6	Dettole	*N.G.*
7	Heptten	*N.G.*
8	Gloves	*Staph epidermis*
9	White coat	*Staph epidermis+ Candida albicans*
10	Light	*Candida albican*
11	*Pressure device*	*Candida albican*

Table (2) microorganisms that isolate from kitchen room

Samples	Results
Nasal Swab	*Corynebacterium spp.*
Nasal Swab	*Staph epidermidi+ Candida albicans s*
Skin Swab	*Staph epidermidis+ Candida albicans*
Table of cafeteria	*E. coli +Bacillus subtilis*
Bowl	*Bacillus subtilis*
Pot	*Pseudomonas aeruginosa*
Table	*Pseudomonas aeruginosa + Listeria ivanovii*
Ground	*Pseudomonas aeruginosa + Corynebacterium spp.*
Wall	*Pseudomonas aeruginosa+ E. coli*
Dining table	*E. coli*
Refrigerator	*E. coli + Lactobacillus spp.*
Refrigerator(Baz)	E. coli
Plate	*Micrococcus varians*
Spoon	*Citrobacter freundii*
Cooker	*E. coli + Corynebacterium spp.*
Plate	*E. coli + Bacillus subtilis*
Glass	*E. coli + Lactobacillus spp.*
Skin swab	*Staph epidermidis*
Nasal swab	*Candida albicans*
Nasal swab	*Corynebacterium spp.+ E. coli*

Table (3) microorganisms isolated from *intensive care ward*

No. of samples	Samples	Results
1	Wall	*Candida albicans + Bacillus subtilisi*
2	Ground	*Candida albicans+ Bacillus subtilisi*
3	table	*Bacillus subtilius &Pseudomonas aerogenosa*
4	Bed	*Micrococcus Varians & Candida albicans*
5	Sacker device	*Staph epidermis*
6	Iodine	*Micrococcus Varians*

Table (4) microorganisms isolated from Labour ward

No. of samples	Samples	Results
1	Ground	*Bacillus subtilius*
2	Wall	*Bacillus subtilius*
3	Articles	*Micrococcus Varians.*
4	Oxygen device	*Lactobacillus spp.*
5	Sacker device	*Enterobacter pnemonia*
6	Balance	*Corynebacterium spp.*
7	Labour table	*Candida albicans*
8	Baby dopplex	*Bacillus subtilis*
9	Nasal swab from staff	*Staph epidermis*

In general we found that Opportunistic bacteria pathogenic or normal flora found or appear with heavy rate in our hospitals and isolate from the major places and articles, and also these bacteria without any doubt cause lethal disease especially to those have immune compromised system, due to the rat of sanitation in our hospitals is too necessary. The four most common types of nosocomial infections are urinary infections, surgical site infection, nosocomial pneumonia, and nosocomial bacteremia. Urinary infections are by far the most common. Eighty percent of these infections are associated with the use of an indwelling catheter. They are associated with less morbidity than other infections but can sometimes lead to septicemia and death. (Ducel, G. *et al*., 2002).

Antibiotic susceptibility for opportunistic isolates: Susceptibility test was conducted for tested isolates; fifteen widely antimicrobials used according microorganism's gram stains as show in table (5). High resistance rates were observed for(RD, Tri, Amp and KF) 100% respectively, CN90% and lower resistance rates were observed for (Cip and Pip) 0%respectivelyand DO 28.57% as show in (figure 1). Resistance has also been found in the absence of antibiotic exposure, the present study is in agreement with (Thaller *et al*., 2010) resistance has also been found in the absence of antibiotic exposure such as in bacteria from wildlife, and persistence of resistant strains under similar conditions.

Table (5) Susceptibility test for Opportunistic isolates

Samples	Amk	Pip	CTX	Amp	Cip	RD	KF	Tri	Tob	CN	Py	E	CX	DO	Ch
Pseudomonas aerogenosa															
6	R	S	R	R	S	R	R	R	S	R	R	-	-	-	R
7	R	S	R	R	S	R	R	R	S	R	R	-	-	-	R
8	R	S	R	R	S	R	R	R	R	R	R	-	-	-	R
Citrobacter sp.	R	S	S	R	S	R	R	R	R	S	-	R	R	S	R
Enterobacter sp	R	S	R	R	I	R	R	R	R	R	-	R	R	S	S
Micrococcus sp.	S	S	R	R	S	R	R	R	R	R	-	R	R	R	R
Bacillus sp.	S	I	R	R	S	R	R	R	S	R	-	R	R	R	R
Staph epidermis env.	S	S	R	R	S	R	R	R	R	R	-	S	R	I	R
Staph epidermis skin	S	S	R	R	I	R	R	R	R	R	-	I	R	S	R
Staph epidermis nose	S	S	R	R	S	R	R	R	R	R	-	S	R	S	S

R= resistant, S= sensitive,I= intermediate, = do not use this antibiotic for this strain

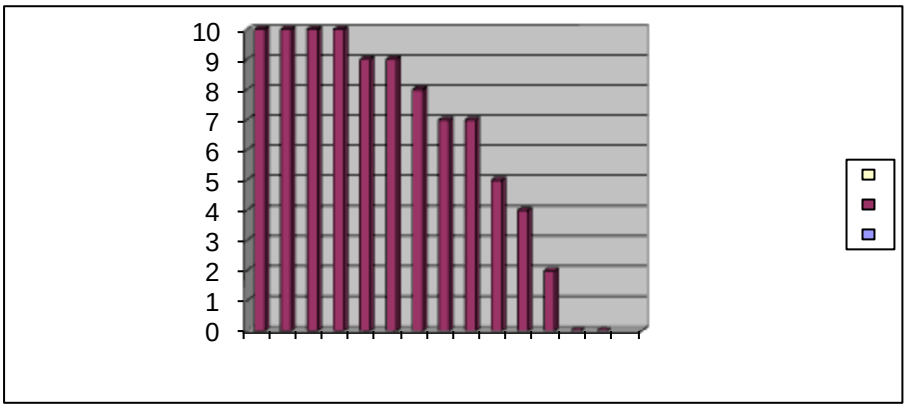

Figure (1) susceptibility test of *opportunistic* isolates to antimicrobials and percentage rate of resistance Column(1- RD100% 2-Tri 100% 3-Amp 100% 4-KF 100% 5- CN 90% 6-CTx 90% 7- Ch 80 % 8- CX100% 9-Tob 70% 10- Amk 50% 11- E 40% 12-DO 28.57% 13-Cip 0% 14-Pip 0%).

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الخلاصة

اجريت هذه الدراسة في مستشفى الشهيد خالد بمدينة كويه وتصمنت عزل(Opportunistic bacteria) من 46 عينة مختلفة لمسحات من عدة مصادر شملت غرفة العمليات، مطبخ، العناية مركزة، صالة الولادات)، أضافة الى مسحاة من جلد وانف العاملين في المستشفى ، و تم الحصول على مجموعة من العزلات . من هذه العينات تم عزل (خمس عزلات لبكتريا ***Micrococcus varians*** وسبع عزلات لبكتريا ***Staph epidermis*** وتسع عزلات لبكتريا ***Escherichia coli*** وعشرة عزلات ***Candida albicans*** وثلاث عزلات ***Lactobacillus sp.*** وخمس عزلات لبكتريا ***Pseudomonas aerogenosa*** وعزلة واحدة لبكتريا ***Listeria ivanovii*** وعزلة واحدة لبكتريا ***Citrobacter freundii*** وتسع عزلات لبكتريا ***Bacillus subtilius*** وعزلة واحدة لبكتريا ***Enterobacter pneumonia*** وست عزلات لبكتريا ***Corynebacteria sp***). تم تشخيص هذه العزلات بالاعتماد على الصفات الزرعية والشكلية والاحتبارات الكيموحيوية . اجريت اختبار حساسية العزلات لخمسة عشرة مضادا حيويا اشتملت هذه المضادات gentamycin (CN), chloramphenicol(Ch) ciprofloxacin(Cip), cefotaximsodium(CTX), amikacin(Amk), pipracillin(Pip), rifampicin(RD), doxycillin (DO), trimethoprim (Tri), pyopein (py), cloxacillin (CX), ampicillin (Amp), erythromycin (E), tobramycin (Tob) and Kiflex (KF). اظهرت العزلات اعلى مقومة بنسبة 100% للمضادات (RD, Tri, Amp and KF) وبنسبة 90% للمضاد CN واظهرت اوطا نسبة من المقاومة للمضادات (Cip and Pip) وبنسبة 0 % وبنسبة .28.57% للمضاد DO .

پوختە

ئەم تاقیکردنەوەیە ئەنجام درا لە تافیگەی کولیجی زانست لە زانکۆی کۆیە، چلو شەش نمونە وەرگیرا لە شوێنی جیا جیا لە (ژووری نەشتەرگەری، لە چێشتخانە، چاودێری وورد، لەگەل ژووری منداڵ بوون)، ئەم جیاکراوانەمان دەست کەوت *Staph epidermis* وە نۆ جیاکراوەی *Escherichia coli* ودە جیاکراوەی *Candida albicans* وە سێ جیاکراوەی یەك *Lactobacillus sp.* وە بێنج جیاکراوەی *Pseudomonas aerogenosa* وە یەك جیاکراوە لە زیندەگی *Listeria ivanovii*وە یەك جیاکراوەی لە زیندەگی *Citrobacter freundii* نۆ جیاکراوەی*Bacillus subtilius* وە یەك جیاکراوەی *Enterobacter pneumonia* وە شەش جیاکراوە لە زیندەگی *Corynebacteria sp*).

لە رێگای ناسینەوەی چاندنو بایو کیمیاوی و وە هەرو هەا دیاریکردنی بەرگری بەکتریاکان بۆ دژە زیندەکیەکان ئەم دژانە بەکار هات (ئەمۆکسیسیلین، ئەمپیسیلین ، سیفۆتاکسیم، کلۆرامفینیکۆل، ئیریترۆمایسین، جینتامایسین، نالیدیکسیك ئەسید، نایترۆفیورانشن، ریفامپسین، ستریپتۆمایسین، تێتراسایکلین، کلیندامایسین، بایبرسلین،گیتفلوکساسین،گلمنتین، تۆبرامایسین و ترایمیتپریم). دەرکەوت کە بەکتریا هەل بەرستەکان زۆر بەرگریان هەیە بۆ دژە زیندەکیەکان 100٪ دژە زیندەکیەکان (RD, Tri, Amp and KF) وە 90٪ بۆ دژە زیندەکي CN وە کەمترین بەرگری بۆ دژە زیندەکیەکانی(Cip and Pip) بە قەدەری 0 ٪ وە 28. 57% بۆ دژە زیندەکي DO.