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Bacterial profile of Infected Traumatic Wound and the Antibiogram of predominant Bacterial Isolates Using Viteck Automated System in Ramadi Teaching Hospital, Iraq.

Shehab A. Lafi[1]; Mahmood Al-Shamarry[2]; Mohamed S. Ahmed[3] and Waleed I. Ahmed[4]
1- Microbiology Dept. College of Medicine University of Anbar, IRAQ .e mail
2- Clinical Lab. Ramadi Teaching Hospital
3- Deputy of Anbar Health Office.
4- Microbiology Dept. College of Medicine University of Anbar, IRAQ.
shehab_6555@ymail.com.

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ABSTRACT

Background: Bacterial pathogens were seen more imposed in wound infections particularly traumatic wounds . Bacterial isolates tend to show high rate of resistance against antimicrobial agents due to bacterial prolonged exposure to such antimicrobial agents in treated patients and gaining of antimicrobial resistance genetic factors and transfer between bacterial generations.

Aims of the study: This study aimed to show the bacterial profile of infected traumatic wounds and the antibiogram of the predominant bacterial isolates.

Patients and methods : Skin swabs were taken from infected wounds of (60 patients were attending Ramadi Teaching Hospital during the period of one year period (From Jan. to Dec. 2017). Bacterial investigations were done for each Specimen aseptically, bacterial isolates were diagnosed using biochemical criteria and confirmed identification was done using Vitec k2 system following. Antimicrobial sensitivity test was done for bacterial isolates using the disc diffusion test method.

Results: It was found that a higher percentage of Gram negative bacterial isolates (42) than Gram positive types (18). *Staphylococcus aureus* took the first rank of isolation (13) followed by *psedumonas aeruginosa, proteus mirabilis* and *klebsiella pneumonia.*, (11,11,10) isolates for each respectively. All bacterial isolates showed good sensitivity to Levofloxacin, Imipenem and Carbapenem while all of them were resistant to Amoxil and Ampicillin with variable resistance ratio to other antibiotics.

In conclusion, different bacterial and antibiotic profiles were found for the isolated bacteria in addition to the high resistance rate to some antimicrobial agents so a continuous periodic study for this category is required.

INTRODUCTION

Wound infection is multifactorial, many types of organisms may be involved in wound infection depending on the wound type (traumatic or surgical) and the site of the wound (Lafi 1997; Vandepitte *et al.*, 2003, Elward 2009, Goyal *et al*., 2013 and kassam *et al.*, 2017).

Many types of bacteria were imposed in wound infection, among of them are MISA and MIRSA *Staphylococcus aureus* (Grundmann *et al.,* 2006, Kim *et al.,* 2010; Goyal *et al*. 2013), in addition to other types of Gram negative bacteria (Lafi, 1997; Giacometti *et al*. 2000). Bacterial type depends on many factors like age of patient , site of the wound, patients gender and personal sanitation (Lafi,1997., Brook 1998., Bowler *et al*. 2001). It was found that infection in different sites of the body show change in microbial pattern, among of them has wound infection (Altemeric 19973, Adoga *et al. 2011*). The response of bacterial isolates to antimicrobial agents is different regarding type of isolate and type of antimicrobial agent and the site of wound and infection (Lafi 1997; Lafi *et al*. 2007, Lautenbach *et al*. 2009, Brooks *et al*. 2013). Abuse of antibiotics in community leads to an increasing resistance of bacteria to antibiotics due to arising of resistance factors like penicillinases of different spectrums (Bradford, 2001, Makena *et al*., 2016). A continuous need for bacterial wound profile and their antibiogram study are needed particularly with abuse of antibiotics communities.

Patients and methods:

The study was done on wound swabs were taken from 60 patients were attending Ramadi Teaching Hospital, Ramadi. West of Iraq during the period of one year (From Jan. Dec, 2017). Each swab was cultivated on blood agar, chocolate agar and MacConkey agar (Oxoid) and incubated aerobically at 37°C for 48 hours, anaerobic blood agar was employed also and cultivated anaerobically using anaerobic gas generating kit (Oxoid). Plates were cultured and examined daily to investigate bacterial growth, bacterial colonies were investigated following (Vandepetti *et al*. 2003, Forbes *et al*. 2007) diagnosed using Viteck 2 System, USA.). The antimicrobial sensitivity test was done for each isolate using Kerby Bauer technique cited by Vandepette *et al*., anti-microbial discs (Biomerix) were used for each antibiotic mentioned in Table (1).

Table 1: types of antimicrobial agents used in antimicrobial sensitivity test .

Type of antimicrobial agent	concentration (µ /Ml)	company
Meropenem (MeM)	30	BioMerieux France
Imipenem (IPM)	12	=
Ciprofloxacin (CiP)	5	=
Rifampicin (RA)	10	=
Gentamicin (GM)	16	=
Amikacin (AN)	16	=
Ceftazidime (CAZ)	8	=
Nitrofurantion (Ni)	300	=
Levofloxacin (LEV)	25	=
Vancomycin (VA)	4	=
Fusidic Acid (FA)	4	=
Augmentin (AMC)	30	AL-Razi Center Iraq
Cephalothin (CeP)	30	=
Ceftriaxone (CTR)	30	=
Ticarcillin (TI)	-----	=
Naladixic Acid (NA)	30	=
Amoxil (Amox)	30	=
Naladixic Acid (NA)	30	=
Amoxil (Amox)	30	=
Naladixic Acid (NA)	30	=

Results were reported and data was analyzed following SSPS system. With plotted Tables and figures .

RESULTS

Gram negative bacterial isolates were showing the bulk (42, 70%) of isolates in contrast to gram positive type (18, 30%) *Staphylococcus aureus* took the first rank of isolation (13) out of 18 gram positive isolates. *Pseudomonas species* and *proteus merabilis* became next, (11) isolates for each. *Then Klebsiella pneumoniae* became next (10) isolates followed by *Acenitobacter bumaneii* were isolated (3) isolates from each gender. Two isolates (2) were recovered from each of E. coli and Enterobacter species, one isolate from each gender was isolated (Tables 2 and 3). Mixed bacterial isolation was found in one swab from male and two swabs from female patients while negative cultivation result was found in specimens from three (3) patients only, two of them were males. Negative cultivation results were found in swab from one male patient and swabs from two female patients (Table 4).

Table 2: Gram positive bacterial isolates.

Type of bacterial isolate	**Male patients**	**Female patients**	**Total number**
Staphylococcus aureus	7	6	13 72.23 %
Staphylococcus epidermidis	1	0	1 5.55%
Staphylococcus hemolyticus	1	1	2 11.12%
Staphylococcus waneri	0	1	1 5.55%
Streptococcus pyogenes	0	1	1 5.55%
Total	9 50%	9 50%	18 100%

Table 3: Gram negative bacterial isolates

Type of Gram Negative Bacterial Isolate	**Male patients**	**Female patients**	**Total Number**
Pseudomonas aeruginosa	7	3	10 23.81 %
Pseudomonas alkaligens	0	1	1 2.38%
Proteus merabilis	6	5	11 26.19%
Klebsiella pneumoniae	5	5	10 23.81%
Acenitobacter bumaneii	3	3	6 14.29%
E. coli	1	1	2 4.76%
Enterobacter cloaca	1	1	2 4.76%
Total	23 54.76%	19 45.24%	42 100%

Table 4: Positive versus negative swab culture

Single isolate			Mixed isolate			No growth		
Male	female	total	Male	female	total	Male	female	Total
27	33	60	2	1	3	1	2	3

Gram Positive Types of Bacterial Isolates:

Eighteen (18), (10.98%) Gram positive bacterial isolates were recovered out of the total number (60) of isolates, nine (9) isolates from each gender. *Staphylococcus aureus* represented the bulk number (13) of isolates, seven (7) recovered were from males and six (6) were from females, so non -significant difference ($p \geq 5$) was found between genders.

Other types of Staphylococcus species became next One isolate of each *Staphylococcus hemolyticus* and *Staphyl-ococcus epidermedis* were recovered from both sexes equally. Only one isolate of Streptococcus pyogenes was isolated from the female patient (Table 2).

Gram Negative Types of Bacterial Isolates:

It included (42, 26.23%) isolates (23, 54.76%) of them were isolated from males and (19, 45.24%) isolates were isolated from females.

Ten (10) isolates were *pseudomonas aeruginosa*, seven (7) of them were isolated from males. One isolate of Ps. alkaligens was isolated from a female patient. *Proteus merabilis* became next, six (6) isolates were from males and (5) isolates were from

females. Five (5) isolates of *Klebsiella pneumonia*were isolated from each gender whilethree (3) isolates of *Acinitobacter bumanei* were isolated from each gender. E. coli and Enterobacter species resembled the lowest isolation rate (one) isolate for each gender equally (Table 3).

Antibiograms of Predominant Bacterial Isolates:

Antibiogram of *Staphylococcus aureus*:

The antibiogram of *Staphylococcus aureus* to used antibiotics revealed that 77.70% of isolated *Staphylococcus aureus*were sensitive to Vancomycine and Ticarcillin followed by Levofloxacin 66.6%. Each of Imipenium, ciprofloxacin and Meropenim were showing 55.5% of sensitivity equally while Augumintine showed the lowest sensitivity (20.3%). All isolates were showing 100% resistance to each of Ampicillin and Amoxil (Figure 1).

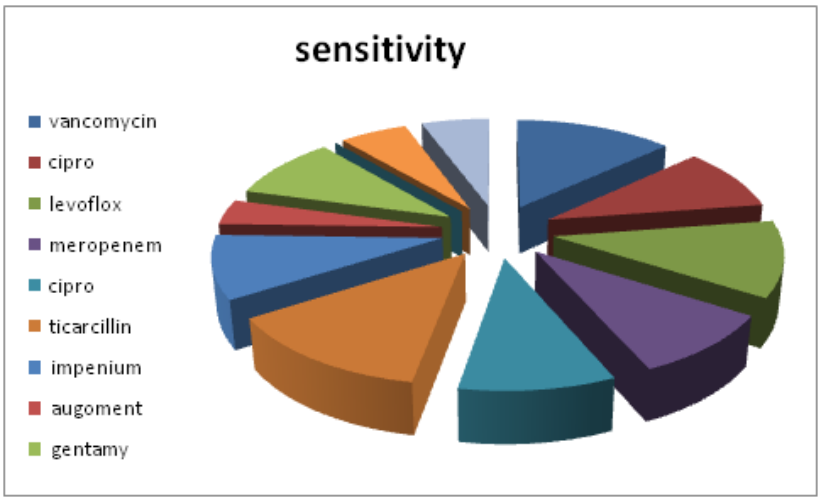

Fig. 1: Antibiogram of Staphylococcus isolates.

Antibiogram of *Psedumonas aerugenosa*:

All isolates (100%) were sensitive to Levofloxacin followed by Imipenem (90%) while 80% of isolates were sensitive to Meropenem, Ciprofloxacin and Amikacin. All isolates were resistant to Refadin and 90% of them were resistant to Cefrtriaxone, Augumentin and Gentamycin (Fig. 2).

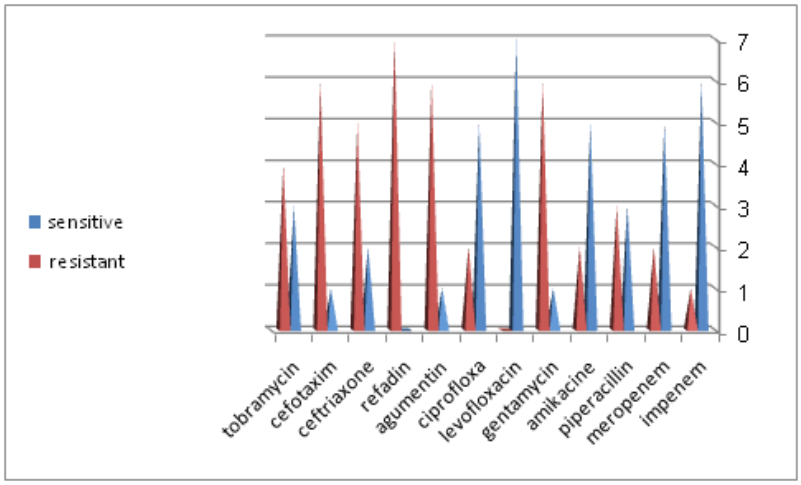

Fig. 2: Antibiogram of *Pseudomonas aeruginosa* isolates.

DISCUSSION

Wound infection is multifactorial it caused by different types of bacteria which cause a reaction in patient, infection can be systemic and the patient becomes ill or local reaction only affecting the wound bed and surrounding tissue (Janda *et al*. 1997, Giacometti *et al*. 2000, Stevens *et al*. 2014).

Ranking of bacterial isolates in this study is different from the study was done in the same hospital on traumatic wounds by (*Lafi* 1997) *who found that Proteus species took the first rank of isolation followed by Pseudomonas aerugenosa and Staphylococcus species this is might be attributed to many factors : among of them are the shift in microbial profile of wound infection during* time (*Altemeier et al*. 1973, *Adogha et al*. 2013) *as well as the arising of more virulent strains of Staphylococcus aureus which are penicillin and methicillin resistant (MIRSA) (Berger-Bachi and Roher* 2002 and even vancomycin resistant VRSA strains (*Smith et al. 1999, Chang et al*. 2003). *These characters enable local isolates of Staphylococcus aureus to be recovered with the highest ratio of isolation.*

In addition to above factors, the carriage rate of Staphylococcus aureus in community may be increased particularly nasal carriage of the organism by normal carriers (Klutmans *et al*. 1997., Wertheim *et al*. 2005., poahl *et al*. 2009).

Staphylococcus aureus showed the highest number of isolation this was attributed to the easy contamination of traumatic wounds with dust and soil bacteria which staphylococcus is included within this group of bacteria. In other hand majority of staphylococcus strains possess high resistance factors like mec a gene for methicillin resistance, Beta-Latamases, Coagulase, Protein A antigen (Bradford 2002, Brook *et al*. 2013, Makena *et al*. 2016) as wells as biofilm formation property makes difficult eradication of this organism difficult (Gotz 2002, Agarwel *et al*. 2010, Otto m., 2013)

In spite of the difference between male and female chances of infection, non-significant difference ($p \geq 0.05$) was found between ratios of bacterial isolation between genders , this was in accordance with a previous study done by (Lafi 1997). This might be due to similarity in chance of affection and stress factors leading to this situation as well as bad hygiene at Ramadi city yielded after ISIS crises at Anbar Governorate during the years 2013, 2014, 2015. Higher number of Gram negative bacterial isolates (43 isolates) than Gram positive bacterial types (18 isolates), this is might be due to difference in wound site and bad community hygiene which give chance for pollution and bad sanitation due to ISIS war and displacement of high number of Ramadi people who came back in 2016 suffering from poor hygiene and low socio-economic status. Non-significant difference between genders in rate of isolation of all types of gram negative isolates was due to the same chance of wound contamination in both sexes due to the above situation and explanation. The same finding was reported by (Kssam *et al*. 2017) who found more Gram negative isolates than Gram positive types with the highest *Staphylococuus aureus* isolation rate.

Staphylococcus aureus predominant isolation rate is due to arising of new resistant strains to Methicillin MIRSA (*Berger-Bachi* and *Roher 2002* Sabol *et al*. 2006., David *et al*. 2008) and low response to other antibiotics even Vancomycin which was considered as therapeutic agent for *Staphylococcus aureus* infections in the Globe (Chang *et al*. 2003., Appelbaum 2006).

The same thing was considered for other bacterial types like *pseudomonas aerugenosa, Kebsiella pneumonia* and *Acenitobacter bumaneii*. Bacteria undergo shift in antimicrobial response because of arising resistant bacteria due production of resistance factors to antibiotics in both gram positive and gram negative bacteria like beta lactamases (Sabol *et al*. 2006, David *et al*.

2008, Al-Okaili 1999) and ability to biofilm formation (Agarwal *et al*. 2010., Otto 2013), so continuous check-up for such category is needed.

Antimicrobial Profiles for Bacterial Isolates:

***Staphylococcus aureus* Profile:**

Vancomycin has traditionally been the antibiotic of choice for MIRSA types of *Staphylococcus aureus* infections. The existence of Vancomycin resistance *Staphylococcus aureus* was reported since 1996 in Japan and USA in 1997 (Hiramastu K. *et al*. 1996, Smith *et al*. 1999) due to possessing of Vancomycine resistance gene known as van A gene (Chang *et al*. 2003) .

Highest rate of sensitivity to Vancomycin (77.7) was observed in this study, this was attributed to low resistance of *local strains of Staphylococcus aureus* to Vancomycin, so it is still applicable antibiotic for the treatment of infections caused by this organism her. Arising of new strains of Vancomycin intermediate resistance strains (VISA) and Vancomycin resistant strains VRSA of MIRSA types of Staphylococci should be continuously explored to choose the alternative antibiotic for treatment (Akosy *et al*. 2008).

Lower sensitivity of isolated staphylococci to other antibiotics mentioned above (Levofloxacin 66.6%. Each of Imipenium, ciprofloxacin and Meropenim were showing 55.5% of sensitivity equally while Augumintine showed the lowest sensitivity (20.3%) this was attributed to the local continuous use of such antibiotics in clinics for treatment of infections caused by MISA and MIRSA . in addition to the abuse of antibiotics in our community by people without prescription by senior doctors., complete resistance to ampicillin and amoxicillin reported her was due to emergence of MIRSA possed mec resistance genes in study area (*Berger-Bachi* and *Roher* 2002 Sabol *et al*. 2006, David *et al*. 2008). The same results were reported by kassam 2017.

Antibiogram of *Psedumonas aerugenosa*:

All isolates (100%) were sensitive to levofloxacin followed by Imipenem (90%) while 80% of isolates were sensitive to Meropenem, Ciprofloxacin and Amikacin. This was might be attributed to the new application of antibotics in Iraq due to previous economic blockade

Before 2003, this leads to delay in import of such antibiotic to the country and making them still acting against psedumonas aeruginosa local isolates. (Al-Okaili 1999, Al-Heety 2013). This was might be attributed to the high antimicrobial reisistance rate of pseddumonas through plasmids.All isolates were resistant to Refadin and 90% of them were resistant to Cefrtriaxone , Augumentin and Gentamycin , this was due to long use of such antibiotics against ps. aeruginosa infections which may leaded to arising of resistance genes of this organism to such antibiotics (Al-Okaili 1999, Al-Heety 2013).

Ps. aeruginosa is one of trouble makers organisms clinically because it possess many virulence factors and has the ability to resist antibiotics due to arising of new resistant strains (brooks 2013, Al-Okaili 1999, Al-Heety 2013)

In conclusion different types of bacteria were imposed in traumatic wounds, both of Gram positive and Gram negative bacteria are accused her. Bacterial isolates showed different profiles to antibiotics tested her due to the difference of these bacterial isolates in its virulence and response (sensitivity and resistance) to antibiotics. So we recommend continuous study of bacterial profile for wound infections both of traumatic and surgical wounds because the profile of infection undergoes difference through years.

Prescription of antibiotics after performing of antimicrobial sensitivity test for the patient to prevent abuse of antibiotics.

Community orientation toward medically controlled antimicrobial therapy and ceasing antibiotics sell without medical prescription .

Continuous and periodic evaluation of microbiological pattern and antibiotic sensitivity of wound infections is necessary to decrease the potential risks of

complications by early institution of appropriate systemic and topical antibiotic as well as Continuous microbial investigation for hospitals and medical care giving centers following biosafety guidelines.

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