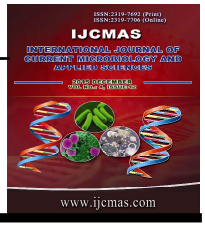
Int.J.Curr.Microbiol.App.Sci (2015) 4(12): 460-472

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 12 (2015) pp. 460-472**
http://www.ijcmas.com



Original Research Article

Prevalence of Antibiotic Resistant Aerobic Bacteria Isolated from Surgical Wounds of Inpatients at Zagazig University Hospitals, Egypt

Magdy.M.M.Bahgat[1], Abdllah.A.Elbialy[2], Mahmoud.M.M.Zaky[1*] and Saly.M.E.Toubar[1]

[1]Botany Department, Faculty of Science, Port-Said University, Egypt
[2]Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, Egypt
**Corresponding author*

Keywords

Antibiotic Resistant Aerobic Bacteria, Surgical Wounds, Vancomycin

A B S T R A C T

Staphylococcus aureus is one of the most common community and nosocomial pathogens and it mainly causes skin and soft tissue infections. So the current study aimed to study the prevalence of VRSA strains in Zagazig University Hospitals. study their antibiotic susceptibility profile and therapy to control and present the transmission of VRSA strains among the health care settings. One hundred pus samples were collected from post operation wounds of a study group of inpatients in Zagazig University-Hospital. Bacteria colonizing the wounds were isolated on specific culture media thereafter purified and divided into 4 groups depending on their morphological characters and Gram's stain reaction. Isolated bacteria were divided into 4 groups. Group (I) represented 38% of total isolates; while group (II) & (III) represented 20% and group (IV) 22%. Isolated bacteria related to groups I, II, III & IV were preliminary identified as *S. aureus. E. coli, K. pneumoniae* and *P. aeruginosa,* respectively. Antibiotic susceptibility of the 4 groups of isolated bacteria against 15 different antibiotics revealed that imipinem and vancomycin are the most effective antibiotics against *S. aureus* group with sensitivity 86.9% and 84.2%, respectively; meanwhile, Imipenem showed highest activity (100%) against *E. coli* and *K. pneumoniae* and 86.3% in case of *P. aeruginosa.* Depending on antibiotic susceptibility pattern some strains were selected as multi-resistant isolates. Strains encoded *S. aureus* 6 &16 were resistant to 11 antibiotics out of 15 (73.3%) and 10 antibiotics out of 15 (66.7%), respectively. the two mentioned strains are methicillin and vancomycin resistant, thus suspected as MRS A and VRSA strains. Imipenem antibiotic as the most effective antibiotic was tested for its MIC and MBC against the selected multi-resistant isolates of the 4 bacterial groups. MICs and MBCs of *S. aureus* 6 & I6 were (62.5 & 31.25) µg/ml and(62.5 & 62.5) µg/ml, respectively. Amplification of 16S rRNA gene of selected multi-resistant strains *S. aureus* (6 &16) revealed the occurrence of amplicons with molecular size 1500bp for both strains. The target van A gene was amplified using Polymerase chain reaction (PCR) in which the primers F: EAl & R: EA2 gave rise to a fragment of about 800 bp size in both tested strains. In conclusion The control of emergence and spread of antimicrobial resistance among the most common human bacterial pathogens is probably one of the most important challenges for the scientific and medical community. This emergence was significantly marked in hospitals that had been endemic with (MRSA) strains and followed the policy of empirical use of glycopeptides. Vancomycin being the cornerstone of treatment of patients with serious MRSA infections for some decades has exerted considerable selection pressure on *S. aureus* strains in the healthcare setting.

Introduction

Wound infections have been a problem in the field of surgery for a long time. An infected wound complicates the postoperative course and results in prolonged stay in the hospital and delayed recovery. Advances in control of infections have not completely eradicated the problem because of development of resistance. Antimicrobial resistance can increase complications and costs associated with procedures and treatment. The wide spread use of antibiotics, together with the length of time over which they have been available have lead to major problems of resistant organisms contributing to morbidity and mortality. Anguzu and Olia (2007).

The primary cause of antibiotic resistance is genetic mutation in bacteria. Also, prevalence of antibiotic resistant bacteria is a result of antibiotic use both within medicine and veterinary medicine Anguzu and Olia (2007).

Bacteria can also resist the effects of antibiotics by enzymatically degrading the drug before it reaches its target site, altering the protein(s) within the bacterium that serve as receptors for the antibiotics, and changing their membrane permeability to the antibiotics (Cloete, 2003).

Antibiotic resistance genes can be transferred between bacteria in a horizontal fashion (between individuals) by conjugation, transduction or transformation. If a bacterium carries several resistance genes, it is called multi-resistant or, informally, a superbug or super bacterium (Chanal *et al*., 2000and Arias and Murray, 2009).

The glycopeptide antibiotic vancomycin was introduced clinically in 1958 for the treatment of gram-positive bacteria. Use of this agent has increased dramatically in the last 20 years, in large part because of the increasing prevalence of methicillin resistance in both coagulase-negative staphylococci and *Staphylococcus aureus* (Srinivasan, *et al.*, 2002).

Vancomycin resistance has been reported in clinical isolates of both coagulase-negative *staphylococci* and *Staphylococcns aureus.* the emerging threat of wide spread vancomycin resistance posses a serious public health concern given the fact that vancomycin has long been the preferred treatment of antibiotic-resistant gram-positive organisms (Srinvasan *et al* ., 2002). .so the current study aimed to study the prevalence of VRSA strains in Zagazig University Hospitals. study their antibiotic susceptibility profile and suggest antibiotic combination therapy to control and present the transmission of VRSA strains among the health care settings .

Materials and Methods

Sample Collection

Hundred medical specimens of pus were collected from patients in different Departments at Zagazig University Hospitals. Samples were obtained during the period from July 2014 to July 2015 from patient suffering from wounds infections.

Isolation and Purification of Bacterial Isolates

Isolation

Samples were collected from wounds infections as pus. Samples were handled by sterile swab. All collected samples were transferred to laboratory within two hours and streaked on nutrient agar to obtain single colony.

Purification

The swabs were streaked on agar surface of different diagnostic and selective media namely, (C.L.E.D agar), Nutrient agar, MacConkey agar , Blood agar and Uri select media.

Plates were incubated aerobically at 37˚C for 24 h. Growing colonies were purified and examined for their systematic position using cultural characters and Gram's stain preparation.

Identification of Bacterial Strains

Media and reagent were prepared according to standard and procedures as described by (Lennette *et al*., 2007).The isolated bacterial stains were identified by the fowlloing tests:

Nitrate reduction, Motility test, Arginine hydrolysis, Gelatin liquification, Oxidase test, Citrate Utilization, Methyl red, Triple sugar iron agar, Catalase activity, Coagulase test.

Antibiotic Susceptibility Test

Antibiotic Disks

Fiften of different antibiotics were selected for carrying out the antimicrobial susceptibility test. Imipenem (IPM), Rifampicin (RF), Tetracycline (TE), Oxycilline (OX), Vancomycin(VAN), Chloromphenicol (C), Cefotaxime(CTX), Cefepime(FEP), Methicllin(MET), Cephardin (CE), Amoxycillin (AX), Ampicillin \ sulbactam (unasyn) (SAM), Ampicillin (AM), Ciprofloxacin (CIP), Erythromycin (E).

The antibiotic disks used in this research were purchased from Oxoid Ltd., England.

Disk Diffusion Agar Method

Antibiotic susceptibility test for the bacterial isolates was carried out by disk diffusion technique according to (Bauer *et al.,* 2003).

Determination of the Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of different antibiotic against selected isolates

Not all the above mentioned antibiotics were tested by this method against different strains including *S. aureus, E.coli, Klebsiella, P.aeruginosa and E.facium* only one antibiotic was used, according to the activity against tested bacterial organism.

Only two antibiotics were used, vancomycin for *S. aureus and E.facium &* imipenem for *E.coli, Klebsiella* and *P.aeruginosa* for purchased from Oxoid Comp. MICs and MBCs were determined by using the standard broth dilution technique (Washengton & Sutter, 2008).

Molecular Characterization of *S. aureus* (6, 16)

PCR 16 Sr RNA and Electrophoresis

DNA extraction and PCR amplification of 16S rRNA region. DNA was isolated from the selected isolates coded 6 and 16 according to (Sambrook et al., 2010).The 16S rRNA was amplified by polymerase chain reaction (PCR) using primers designed to amplify 1500 bp fragmem of the 16S rRNA region. The forward primer was 5'AGAGTTTGATCMTGGCTCAG3' and the reverse primer was 5'TACGGYTACCT TGTTACGACTT3'.

The PCR mixture consists of 30 picomoles of each primer, 10 ng of chromosomal

DNA. 200 µM dNTPs and 2.5 Units of Taq polymerase in 50 µl of polymerase buffer. The PCR was carried out for 30 cycles in 94˚C for 1 min. 55˚C for 1 min and 72˚C for 2 minutes. After completion, a fraction of the PCR mixture was examined using agarose gel electrophoresis (Ausuble *et al*., 2009) and the remainant was purified using QIA quick PCR purification reagent (Qiagen)

Polymerase Chain Reaction (PCR)

The PCRs were carried out in order to amplify specific DNA fragments (Mullis *et al.,* 2006). For standard PCR, The PCR mixture consisted of 25 pmol of each primer, 10 ng of DNA, 20 µM dNTPs,, MgCl2, 2µM and 2.5 U of Taq polymerase in 50µl polymerase buffer final concentration1 x. The cyclic reaction composed of 4min at 94˚C; and then 30 cycles of 1 min at 94˚C, 1 min at 48˚C and 2 min at 72˚C, followed by additional 10min at 72°C. The optimal parameters, and the annealing temperatures in particular, had to be determined empirically depending on the fragment to be amplified. For standard PCR the optimal annealing is 55˚C.

Results and Discussion

Isolation of Bacteria Colonizing Post Operation Wounds of Inpatients at Zagazig University Hospitals

One hundred pus samples were collected from post operation wounds of inpatients in different departments at Zagazig University Hospital. The study group included 55 males and 45 females (table 1). Bacteria colonizing the wounds were isolated on specific culture media thereafter purified and divided into 4 groups depending on their morphological characters and Gram's stain reaction.

The 4 groups included Gram +ve Staphylococci(I). Gram -ve non motile rods growing on ordinary media(II). Gram -ve non motile rods growing on ordinary and MacConkey media(III). Gram -ve motile rods growing on ordinary and MacConkey media (IV).

Bacteria related to Group I represented 38% of total isolates: while group (II)& (III) represented 20% and group (IV) 22% (Table 3 & 4) illustrated by fig(l).

Antibiotic Susceptibility of the 4 Groups of Bacteria

Antibiotic Susceptibility of *S.aureus* Strains

Susceptibility of 38 strains of *S.aureus* to 15 different antibiotics was examined. Data in tables (6&7) revealed that imepenim and vancomycin arc the most effective antibiotics against the tested strains where 86.9 & 84.2% of tested strains were sensitive : 5.2&2.7% intermediate and 7.9 & 13.1% resistant, respectively. Obviously. 63.2% of total *S. aureus* group showed recognizable resistance to methicilin and thus defined as MRSA strains and 13.1% (5 isolates) resistant to vancomycin and suspected as VRSA strains. Meanwhile, ciprofloxacin(CIP) was the following effective antibiotic against 65.8 % of tested strains (25 out of 38).

Determination of Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentration (MBCs) of Imipenem Antibiotic against Selected Multi-Resistant Strains

Minimum inhibitory concentration is the lowest concentration that inhibits the growth in liquid media while the minimum bactericidal concentration is defined as the lowest concentration that inhibits the growth in solid media.

Imipenem antibiotic was observed to be the most effective antibiotic against the 4 tested bacterial groups isolated from the collected pus samples. Thus, it was important to determine the MIC and MBC of this antibiotic against the selected multi-resistant isolates of the 4 bacterial groups.

Multi-resistant isolates were treated separately with double fold concentrations of the antibiotic under test in nutrient broth and on solid agar medium. MIC of imipenem against *E. coli* 5 and *K. pneumoniae* no 22 was 31.25 µg/ml and MBC for bolh isolates was 62.5 µg/ml. Concerning *P. aeruginosa* no 54. the recorded MIC and MBC were 62,5 and 125 (µg/ml, respectivly. MICs and MBCs of *S. aureus* 6 &16 were (62.5 & 31.25) µg /ml and 62.5 & 62.5 µg/ml, respectively (table 18). Results revealed that *S. aureus* 6 and *P. aeruginosa* 39 showed highest resistance to imipenem antibiotic.

The obtained values of MICs and MBCs of vancomycin against resistant strains of *S. aureus* (6,16, 26, 27, 62 & 63) are recorded.

Results revealed that MICs of *S. aureus* (26, 62, 63) were 31.25 µg/ml and those of *S. aureus* (6 & 16) were 62.5 µg/ml and *S. aureus* (27) were 15.625 µg/ml, respectively.

Determination of Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of Ciprofloxacin(CIP) Antibiotic for Multi-Resistant *S. aureus* 6&16 Strains.

Ciprofloxacin being effective e against tested *S. aureus* strains with 65.8 % effectiveness was examined for its MIC and MBC. Recorded values of MICs and MBCs are 62.5 µg/ml for *S. aureus* 6&16 and 125 µg/ml, respectively.

Determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of Cefipeme acid antibiotic against multi-resistant *S. aureus* .

Studying the effectiveness of cefipeme on studied multi-resistant *S. aureus* 6&16 strains showed that MICs values were 125 & 62.5 µg/ml for *S. aureus* 6 & 16, respectively. Meanwhile, MBCs values were 125 µg/ml for both strains

Determination of Minimum Inhibitory (MIC) and Minimum Bactericidal Concentration (MBC) of Oxicillin Antibiotic for Multi- Resistant *S. aureus* 6&16

Data in table (4) revealed that MICs ofoxacillin against *S. aureus* 6 & 16 were slightly higher than previously tested antibiotics reflecting its low-effectiveness. Recorded values were 125 µg/ml for MICs against *S. aureus* 6&16 and (250& 125 µg /ml) for MBCs, respectively.

Amplification of 16S rRNA Gene of Selected Multi-Resistant *S. aureus* (6 &16)

The two selected *S. aureus* (6 & 16) isolates suspected as VRSA strains previously identified according to their phenotypic characters were confirmed for their identity by amplifying their 16S rRNA genes. The amplification was carried out using specific universal forward and reverse primers EA 1 &. EA2 (their sequences mentioned in materials and methods. Agarose gel (photo2) revealed that the occurring bands of both amplicons matched DNA ladder marker at 1500 bp confirming them as *S. aureus* strains.

Table.1 Determination of Minimum Inhibitory Concentration (MICs) and Minimum Bactericidal Concentration (MICs) of Imipenem Antibiotic against Selected Multi-Resistant Strains

NO	Isolate	MlC(µg/ml)	MBC (µg/ml)
l	***E. coli* 5**	31.25	62.5
2	***K. pneumoniae* 22**	31.25	62.5
3	***P. aeruginosa* 39**	62.5	125
4	***S. aureus 6***	62.5	62.5
5	***S. aureus 16***	31.25	62.5

Table.2 Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of Vancomycin Antibiotic against Resistant *S. aureus* Strains

No	Isolate	MIC µg/ml	MBC µg/ml
1	***S. aureus* 6**	125	250
2	***S. aureus* 16**	62.5	125
3	***S. aureus* 26**	11 .25	67.5
4	***S. aureus* 27**	15.625	15.625
7	***S. aureus* 62**	31.25	31.25
8	***S. aureus* 63**	31 .25	62.5

Table.3 MICs and MBCs of Cefipeme against *S. aureus* 6&16

No	Isolate	MIC µg/ml	MBC µg/ml
1	***S . aureus* 6**	125	125
2	***S. aureus* 16**	62.5	125

Table.4 MICs and MBCs of Oxicillin Antibiotic against Multi- Resistant *S. aureus* 6 and 16

No	Isolate	MIC µg/ml	MBC µg/ml
1	***S. aurens 6***	125	250
2	***S. aurens 16***	125	125

Fig.1 Pie Chart Showing Frequency of Bacterial Isolates within Different Groups

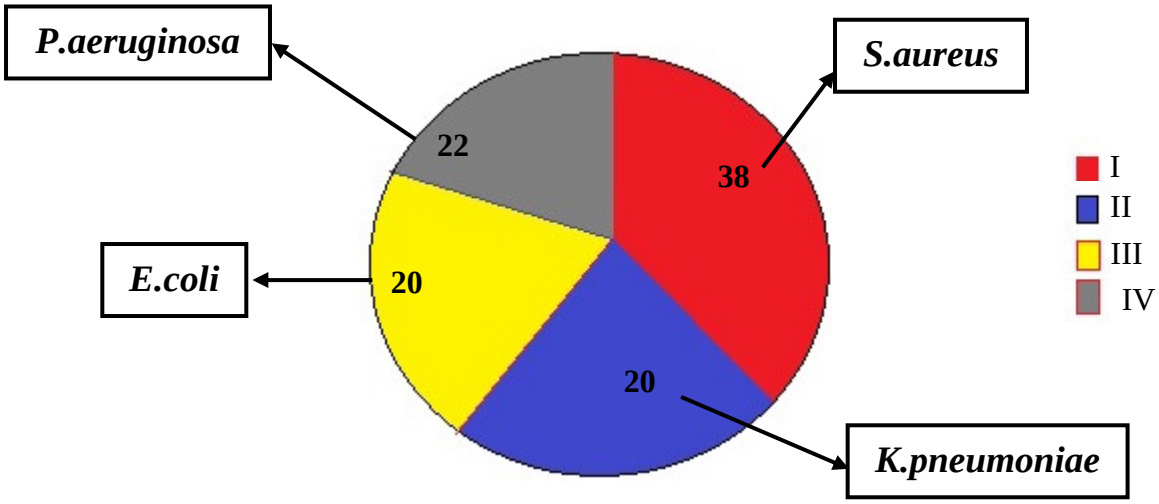


Fig.2 Antibiotics Resistance Pattern of *S. aureus* Strain

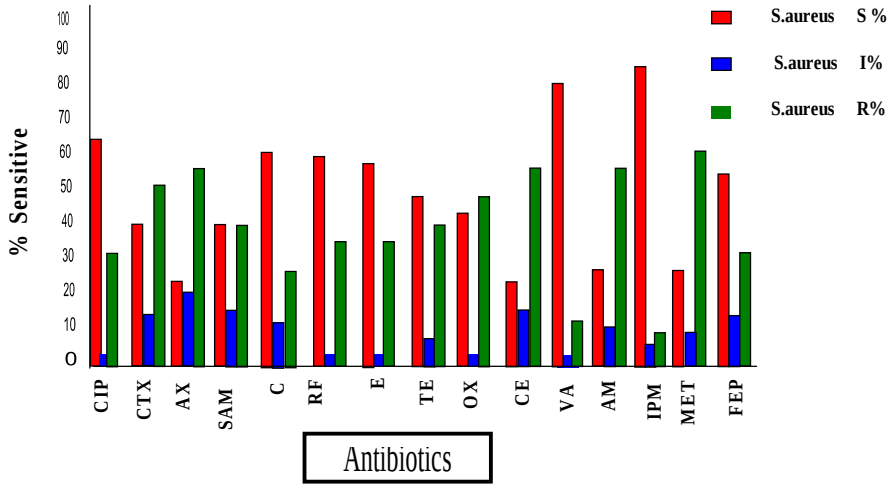


Fig.3 Resistance % of *S. aureus* Strains to (15) Tested Antibiotics

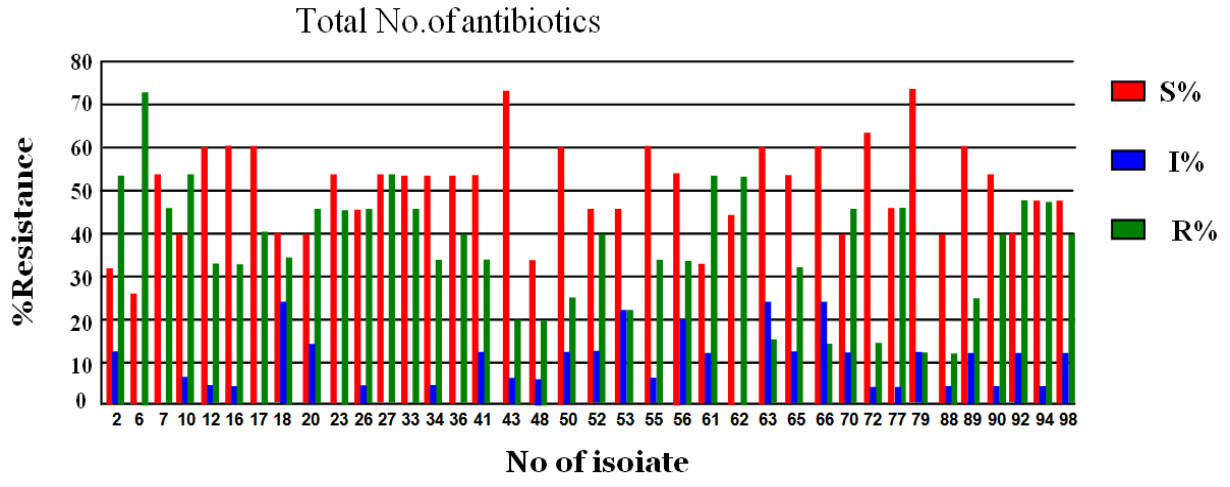


Fig.4 MICs and MBCs of Imipenem Antibiotic against Multi-Resistant Strains

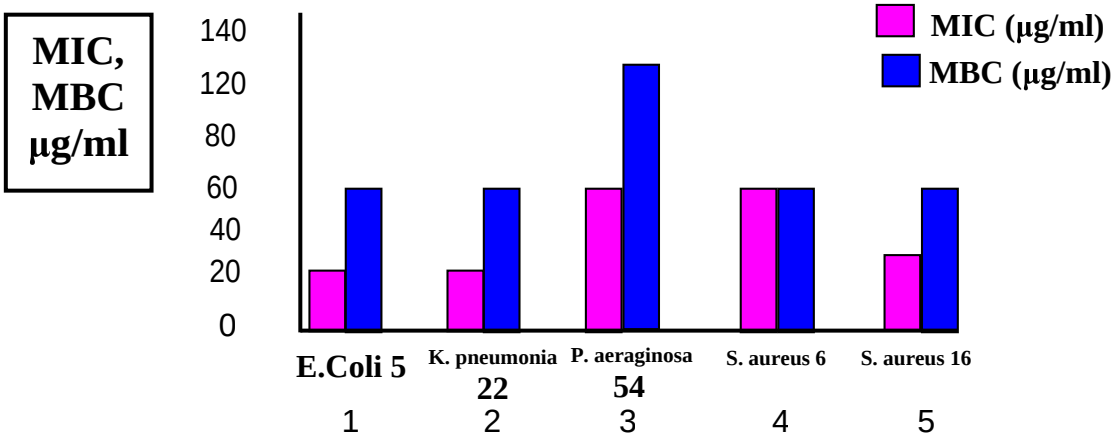


Fig.5 Determination of MICs and MBCs of Antibiotic against Multi-Resistant *S. aureus* Strains

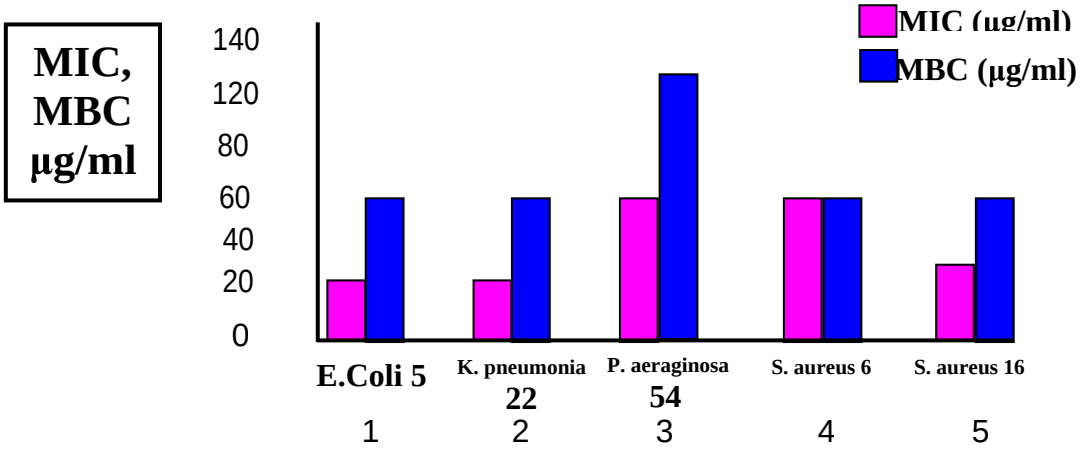
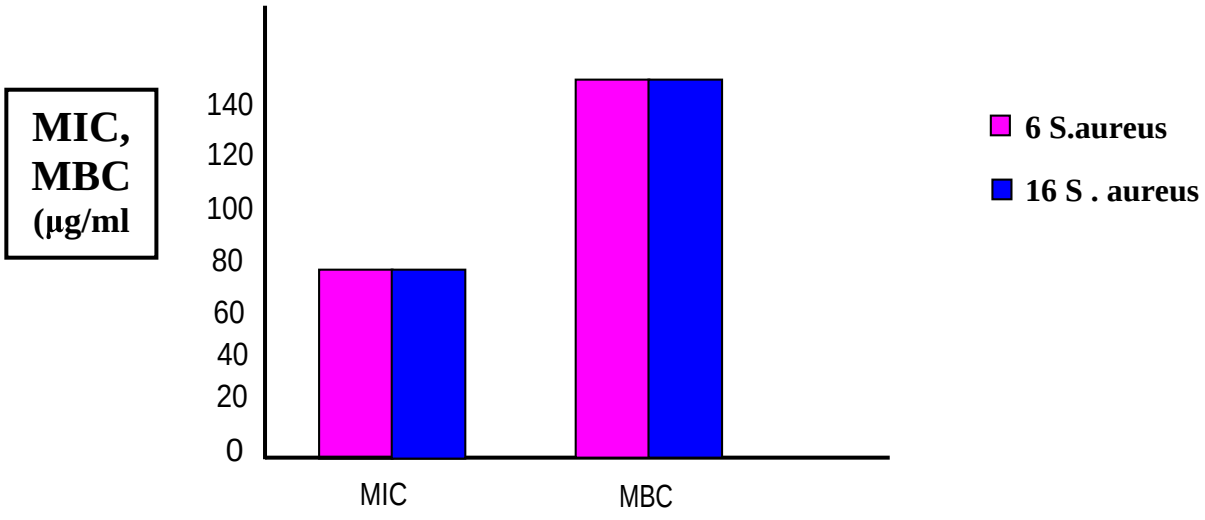


Fig.6 MICs and MBCs of Ciprofloxacin Antibiotic Against Multi-Resistant *S. aureus* 6 and 16 Strains



Amplification of Target Van A Gene using Polymerase Chain Reaction (PCR)

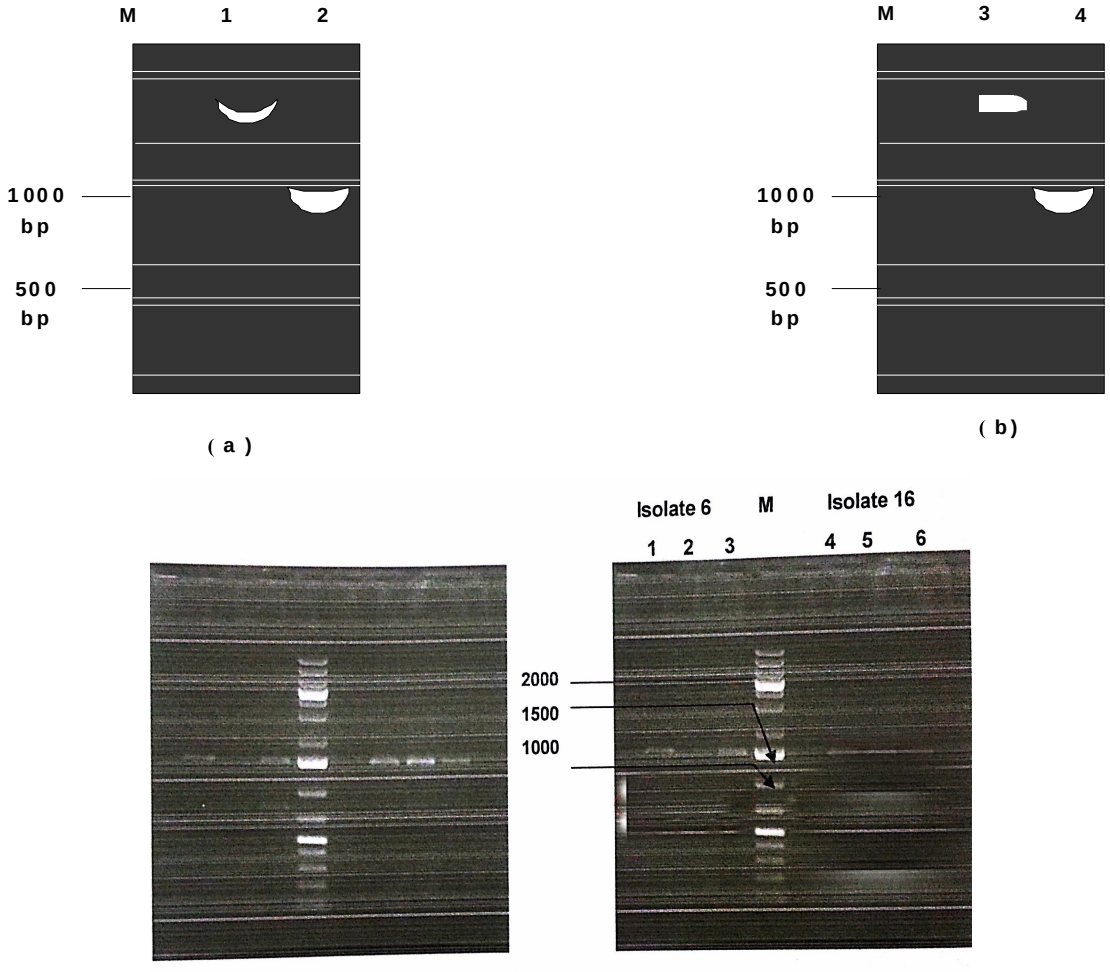


Photo (3): Agarose gel electrophoesis showing van A gene in the two tested strains with molecular size 800bp. Lines 1,2,3: PCR products using specific primer for strain AB674510 (ZAE 6). Lines 4, 5, 6: PCR products using specific primer for strain AB674511(ZAE 16). M: DNA 100 bp ladder.

Surgical wound infection is a serious hazard to patients, with incidence according to US Centers for disease Control (CDC) was 15.45% and according to the UK. nosocomial infection surveillance was 11.32%. and according to ASEPSIS was 8.79% (Ashby *et al*., 2010). About 77% of the deaths of surgical patients were related to surgical wound infection (Mangram *et al*., 2009).

Bacterial agents often incriminated with wound infections include *Staphylcoccus, Klebsiella, E. coli* and *Proteus* as well as anaerobs as *Clostridium* and *Bacteriodes* species the most common group of bacteria responsible for SSIs is *Stuphyloccus aureus* (Chastre and Trouillet, 2000).

The present study was initiated by collection of hundred specimens of pus from wounds of inpatients in different departments at Zagazig University Hospital, Zagazig, Egypt.

Bacterial isolates were purified and initially identified depending on morphologic characteristics and Gram's stain reaction. They were divided into 4 main groups; group I included 38 Gram positive bacterial isolates (38%) preliminary identified as *Staphylococcus aureus* . Groups II, III & IV included Gram negative bacterial isolates (62%) related to *E. coli* (20%), *K. pneumonsae* (20%) and *P. aeruginosa* (22%), respectively.

Yamaguchi *et al*., (2002) reported that Gram-positive cocci predominate as a cause of nosocomial and community acquired infections. Nutanbala *et al.,* (2005) upon studying bacteria contaminating surgical wound infections observed Chat 31.15% pathogens were Gram positive and 68.85% were Gram negative. Meanwhile, in other setups they observed that 69% for gram positive and 29% for Gram negative organisms. Previous studies observed that though percentage of Gram negative bacilli from the wounds was more, *Staphylococcus aureus* was predominant organism isolated followed by *Klebsiella pneumoniae Pseudomonas aeruginosa* . and *Escherichia coli* (AbuHanifah, 2001 ; Giacometti, 2000 :Malone *et al.,* 2002, Thanni *et al.,* 2003 and Surucuoglu *et al.,* 2005). Mulu *et al*., (2006) reported that bacterial pathogens isolated from 79 patients showed an isolation rate of 52% in which *S.aureus* was the predominant species 65% (51/79) followed by *E.coli,* 8/79 (10%), *K.pneumoniae* 9% (7/79), *Proteus* species 4% (3/79) and Streptococci species 4% (3/7). The organisms most frequently involved in surgical infections change from time to time, and also vary with hospital sellings. This difference may be due to variation in common nosoeomial pathogens inhabitant in different hospital set up.

In this study the antibiotic susceptibility pattern of 100 bacterial isolates to 15 different antibiotics was investigated by using disc diffusion method. The included antibiotics were: IPM, ClP, CTX, AX, SAM, C, RF, E, TE, OX, CE, VAN, AM, MET and FEP. Results revealed varying response of bacterial isolates to the investigated antibiotics. Data showed that Gram negative isolates collected from different source (62 isolates) were resistant to AX, RF,OX,VAN, AM, and MET with percentage between 45 to 85%. On the other hand, Gram positive isolates (38%) showed lower sensitivity to the tested antibiotics between 7.9 to 63.2.

In a similar manner, Giacommetti *et al.,* (2000) observed very low. susceplibility rate (10%sensitivity) for ampicillin and amoxicillin against all Gram positive and Gram negative bacteria isolated from surgical wound infections. In addition Zaid (2001) reported that the resistance of 94 strains of *P. aeruginosa* and *E. coli* studied to 12 β-lactam antibiotics and found that all tested isolates were resistant to at least 7 β-lactam antibiotics (penicillin, ampicillin, amoxicillin, amoxicillin/clavulanic acid, cepalexin. cephardin, and cloxacillin). Moreover Yamaguchi *et al.,* (2002) reported that antibiotic resistance analysis showed that all tested *E. coli* strains were highly resistant to ampicillin, piperacillin, carbenicillin, cephaloridine, and cefotaxime; intermediately resistant to cefoxitin; moderately susceptible to moxalactam and ceftazidime; and susceptible to imipenem. Srinivasana *et al.,* (2007) found that all tested E. *coli* strains were resistant to two or more antimicrobials used in veterinary medicine including ampicillin, furadantine, cetazidime and cefotaxime. El-Aidy (2008) observed that pencillin G and ampicillin showed low activity against all tested Gram positive and Gram negative isolates.

Results showed that imipenem proved to have a broad spectrum and high activity

against all the tested Gram positive and Gram negative bacterial isolates. The susceptibility rates of imipenem for *S. aureus, E. coli , K. pneuiuaniae, P. aeruginosa* were 86.9,100,100 & 86.3%. respectively. hese results are in accordance with data reported in other studies of (Gowan *et al.*, 2009; Amyes and Gemmell, 2007).

Multidrug resistance (MDR) is emerging problem in the clinical management of bacterial infections continues to challenge the healthcare sector. In particular, multidrug resistance is now common in familiar pathogens such as *S. aurues* and *Mycobacterium tuberculosis* as well as emerging pathogens such as *Acinetobacter baumannii.* New antibiotics and new therapeutic strategies are needed to address this challenge (Gerard and Arlene, 2007). Enterobacteriaceae isolates resistant to multiple antibiotics have been reported from several purls in the world. (Hall *et al*., 2003). *P. aeruginosa* is an opportunistic pathogenic bacteria which is usually very hard to control by antibiotic therapy.

Obviously, this study revealed that 63.2% of total *S. aureus* group showed recognizable resistance to methicillin and thus defined ,as MRSA strains and 13.1% (5 isolates) resistant to vancomycin and suspected as VRSA strains.

More than 90 % of *Staphyhcoccus* strains are resistant to penicillin (Chambers, 2001), followed by increasing resistance to methicillin, aminoglycosides, macrolides and lincosamide.

In the present investigation, MICs and MBCs of Vancomycin. Imipenem, Ciprofloxacin, Oxacillin and Cefipeme antibiotics against the selected multi-resistant isolates of *S. aureus* (6 and 16) cultivated in liquid media were determined visually. The results showed *S. aureus* (6, 16) more resistant to OX than FEP The MICs and MBC of VAN were 125 & 250 µg/ml for *S. aureus* (6) while those for *S. aureus* (16) were 62,5 & 125 µg/ml for, respectively. MICs and MBCs of IPM were 62.5& 62.5 µg/ml for *S. aureus* (6) and 31.25& 62.5 µg/ml for *S. aureus* (16), respectively. Also, the MICs and MBCs of CIP were 62.5 & 125 µg/ml for *S. aureus* (6) and 62.5 & 125 µg/ml for *S. aureus* (16), respectively. Concerning OX, the values were 125& 250 µg/ml for *S. aureus* (6) and 125 & 125 µg/ml for *S. aureus* (16), respectively. In case of FEP recorded values for *S. aureus* (6) were 125 & 125 µg/ml 62.5 & 125 µg/ml for *S. aureus* (16), respectively.

In the present investigation two isolates *S. aureus* 6 & 16 which were isolated from wounds and selected as multi-resistant to most tested antibiotics were selected for further investigations. Both isolates (6 and 16) were preliminary identified according to as *Staphylococcus aureus.* PCR amplification of 16s rRNA gene of the two selected strains using specific primers produced amplicons of size 1500bp for both strains. PCR products confirmed the identity of the two strains as *S. aureus.* The partial nueleotide sequences of 16S rRNA gene of the two tested strains named *S. aureus 6* (ZAE 6)and *S. aureus* 16 (ZAE 16) were then submitted to the DDBJ/EMBL/ GenBank with the accession number(s) AB674510 and AB674511, respectively.

In the current study, synergy was observed upon combination between Van and 1pm antibiotics in all tested ratios. The least values of MICs and MBCs (15.125 to 31.25 µg/ml) were recorded in case of mixing Van and Imp in ratio l:l(v/v). Similarly, mixture of Van and Cip antibiotics in ratios 1:1 &

3:1 decreased MICs and MBCs to about half their values in case of separate treatments. Meanwhile, no significant changes in MICs and MBCs values were observed in case of combination between Van and Ox or Van and Cip antibiotics.

The PCR amplification of van A gene in both tested strains *S. aureus* [AB674510 & AB674511] using the gene specific primers yielded a 800 bp amplicon as expected and thus the two strains were confirmed as VRSA strains.

References

Anguzu, J.R. and Olila, D.(2007): Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a referral hospital in Uganda.Afr Health Sci. 7(3): 148-154.

Cloete, T. E. (2003): Resistance mechanisms of bacteria to antimicrobial compounds. Intl. Biodeter. Biodegrad., 51:277-82.

Chanal, C.; Sirot, D.; Romaszko, J. P.; Bret, L. and Sirot, J. (2000): Survey of prevalence of extended spectrum (3-Lactamases among exterobacteriaceae. J. Antimicrob. Chemother. 38:J27-132.

Srinivasan, A.; Dick and Trish M.(2002):Vancomycin Resistance in *Staphylococci* Clin Microbial Rev. 1 5(3): 430-138.

Lennette, E. H.; Spaulding, B. H. and Truant, .J. B. (2007):Manual of clinical microbiology 2nd (ed). (AMSPress), Washington D.C.

Bauer, A.W.; Kirby, W.M.; Sherries, J.C. and Truck, M. (2003): Antibiotic susceptibility testing by the standard single disk method, J.Clin. pathol.,45:493-496.

Washington, J. A. and Sutter, V. L. (1980): Dilution susceptibility test, Agar and macrobroth dilution procedures. J. Med. Microbiol. 10: 453-458.

Ausubel F. M., R. Brent, R.E. Kingston, D.D. More, J.G. Seidam, J.A. Smith and K. Struhl (eds). (2009): Short protocols in Molecular Biology. JohnWilley and Sons, Inc. NY.

Ashby E, Haddad FS, O'Donnell E, Wilson AP (2010) : How will surgical site infection be measured to ensure "high quality care for all"? J Bone Joint Surg Br 2010 ; 92:1294-9.

Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR.(2009): Guideline for prevention of surgical site infection, 3999. Hospital Infection Control Practices Advisory Committee. Infect Control Hosp Epidemiol 2009 ;20:250-78.

Chastre J, Trouillet.JL (2000): Problem pathogens *(Psendomonas aeruginosa* and Acinetobacter). Semin Respir Infect 2000; 15:287-98 .

Yamagishi, K.; Matsuo, H. and Ishii, Y (2002): Characterization of cefotaxime-resistant *Escherichia coli* isolates from a nosocomial outbreak at three geriatric hospitals. J. Clin. Microbial. 50: 663-668.

Nutanbala N Goswami, Hiren R Trivedi, Alpesh Puri P Goswami, Tejas K Patel, CB Tripathi. (2005):Antibiotic sensitivity profile of bacterial pathogens in postoperative wound infections at atert.

Abu-Shady, M. R ; EI-Beih, F. M. and El-Satoury, E. H.(2001): Total lipid and lipid pattern of certain antibiotic resistant and sensitive strains of *E. coli and Pseudomonas aeuroginosa.* Egypt Microbial., 9:37-50.

Giacommetti, A.; Ciriani O.; Schimizzi, A.M.; Del prete M.S.; Barchiesi,F.; Derrico M, M.; petrelli E. and Scalise G. (2000): Epidemiology and Microbiology of surgical wound infections. J. Clin. Microb. 38(2): 918.

Malone D L, Genuit T, Traey, JK, Gannon C, Napolitano LM (2002):Surgical site infections. Reanalysis of risk Factors. J Surg Res 2002; 103:89-05.

Thanni LO, Osinupebi OA, Deji-Agboola M.(2003):Prevalence of bacterial pathogens in infected wounds in a tertiary hospital, 1995-2001: any change in trend?J. Natl Med Assoc 2003;95:1189-95.

Surucuoglu, S., Gazi, H., Kurutepe, S., Ozkutuk, N. and Ozbakkaloglu, B.(2005): Bacteriology of Surgical Wound Infections in a Tertiary Care Hospital in Turkey. East Afr. Med . J., 82(7): 331-336.

Mulu A, Moges F, Tessemn B, Kassu A. (2006): Pattern and multiple drug resistance of bacterial pathogens isolated from wound infection at University of Gondar Teaching Hospital, North West Ethiopia. *Ethiop Med* .J. 44(2): 125-131.

Zaid, A. M. (2001): Studies on β-lactamascs producing bacteria belonging to genus *Pseudomonas.* Ph.D. thesis. Faculty of science, Zagazig Univ.

Srinivasan, A.; Dick and Trish M.(2002):Vancomycin Resistance in *Staphylococci* Clin Microbial Rev. 1 5(3): 430-138.

El-Aidy, E. E. G. F. (2008): Antibiotic resistance of some microorganisms isolated from cancer patients. M.Sc thesis, faculty of Science, Zagzig Univ., Egypt.

Gowan, J. F., Jr Hall, E. C. and Parrott, P. L. (2006):Antimicrobial susceptibility in Gram-negative bacteremia are nosocomical isolates more resistant. Antimicrob Agents Chemother. 39 (11) : 645-649.

Amyes, G. B. and Gemmell, C.G. (2007): Antibiotic resistance. J. Med. Microbial. 46 (2): 436-470.

Gerard, D. W. and Arlene, D. S. (2007): New strategies for combating multidrug-resistant bacteria J. Appl. Microbial., 13(6): 260-267.

Hall, L. M.; Blok, M.; Dondcrs, T.; Paauw, A.; Fluit, A. C.andVerhoef, J. (2003): Multidrug resistance among Enterobacteriacae is strongly associated with the presence of integrons and is independent of species or isolate origin. J. Infact. Dis., 187(2): 251-259.

Chambers, H. F. (2001): The changing epidemiology of *Staphylococcus aureus?* Emerg Infect.