NOSOCOMIAL (HOSPITAL) INFECTION WITH SPECIAL REFERENCE TO GENOTYPING OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA): A HIGH RESOLUTION TECHNIQUES AND SPECIFIC MARKERS TO IDENTIFY SOURCES AND MODES OF INFECTION

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And General Surgery Dept**

ABSTRACT
Nosocomial infection is the infection acquired within a hospital in a patient hospitalized for at least 72 hours without signs or symptoms of infection prior to this time. It is one of the major problems all over the world concerning the patients and every member in hospital. The continuing increase in the incidence of these infections represents an important problem in many countries, since they are associated with a high degree of morbidity and mortality.

Aim of the work: This study was conducted to estimate the prevalence of pathogens in different type of N.C.I with special reference to MRSA as a causative agent of nosocomial infection. In addition, genotyping of the isolates will be done to add precise markers for the epidemiological investigations of MRSA.

Patients: This study was conducted over a period of eight months starting from October 1998 till the end of May 1999 on all patients (36700) admitted to Mansoura University Hospital. Patients included in this study are those who acquired infection in the hospital after admission i.e. nosocomially infected patients (1296 patients).

Patients were grouped according to the site of nosocomial infection into
four groups.
1- Patients with post operative wound infection (POWI). 2- Patients with bacteremia.
3- Patients with lower respiratory tract infection (LRTI). 4- Patients with urinary tract infection (UTI).

Methods: I- Bacteriological study
[1- Samples collection and transport
2- Culture 3- Identification of growth
4- Antimicrobial susceptibility testing]
II) Genotyping [1- Chromosomal DNA extraction 2- Digestion of chromosomal DNA]

Results: Nosocomial infection rate during the period of study was 3.6% different types of nosocomial infections were POWI (47.2%), Bacteremia (19.2%), LRTI (11%), UTI (22.6%). Distribution of MRSA according to the type of nosocomial infection. POWI were the most prevalent and represented (45.7%), both LRTI, bacteremia represented (20%) and UTI represented (14.3%). All MRSA isolates in surgical and medical patients were sensitive to rifampicin and vancomycin. Frequency of MRSA isolates genotypes were discriminated into 8 different genotypes, these were given letters from (A to H). All genotypes were sensitive to Vancomycin and Vancomycin and

Rifampicin

Conclusions: NCIR represented (3.6%). The most common NCI was POWI (47.2%) followed by UTI (22.6%), bacteremia (19.2%) and lastly LRTI (11%). Pseudomonas aeruginosa was the commonest pathogen in POWI (20.5%) and Staph. aureus was the commonest pathogen in nosocomial bacteremia (20.9%). Nosocomial MRSA infection represented (2.7%) of the total nosocomial infections and were more prevalent in surgical patients than in medical patients. Vancomycin and rifampicin were most effective (100%) followed by imipenem (75%). MRSA isolates were divided into 8 groups depending on the pattern of digestion with the restriction enzyme Bgl II. Type A was the most prevalent (20%) and type G was the least prevalent (2.9%). Analysis of MRSA isolates from various hospital outbreaks by genotypic fingerprinting techniques can be used to identify transmission routes and reservoirs of MRSA clones in the hospital environment.

INTRODUCTION
Nosocomial infection is the infection acquired within a hospital in a patient hospitalized for at least 72 hours
without signs or symptoms of infection prior to this time. It is one of the major problems all over the world concerning the patients and every member in hospital (Davies et al., 1992).

Staphylococcus aureus is a versatile human pathogen that continues to be an important cause of nosocomial infection (Vanhoof et al., 1994).

Methicillin-resistant strains of Staph. aureus (MRSA) were recognized soon after the clinical introduction of methicillin. In 1982 epidemic MRSA strains showing multiple antibiotic resistance and enhanced capacity to cause wide spread outbreaks of infections were described (Vanhoof et al., 1994).

The continuing increase in the incidence of these infections represent an important problem in many countries, since they are associated with a high degree of morbidity and mortality (Cookson and Phillips, 1988).

All these finding justify surveillance programs in which specific epidemiological markers are used to type the strains and to identify major sources of infection and the main route of dissemination (Vanhoof et al., 1994).

Analysis of MRSA isolates by genotyping fingerprinting techniques has begun to provide the first insight into the evolution of bacterial clones in the in vivo environment (Boyce et al., 1993).

In addition, these methods provide also high resolution techniques that can be used to identify mode of transmission (De lencastré et al., 1996).

Aim of the work: This study was conducted to estimate the prevalence of pathogens in different type of N.C.I with special reference to MRSA as a causative agent of nosocomial infection in Mansoura University Hospital (MUH) In addition, genotyping of the isolates will be done to add precise markers for the epidemiological investigations of MRSA.

PATIENTS AND METHODS
This study was conducted over a period of eight months starting from October 1998 till the end of May 1999 on all patients (36700) admitted to Mansoura University Hospital.

Patients included in this study are those who acquired infection in the hospital after admission i.e. nosocomially infected patients (1296
patients).

They were diagnosed from different wards regardless the sex and age with the following precautions:

- No pre-admission infection (proved by history and clinical examination that revealed no signs or symptoms of infection.
- The patients developed signs and symptoms of infection three days after the admission.

Patients were grouped according to the site of nosocomial infection into four groups.
1- Patients with post operative wound infection (POWI).
2- Patients with bacteremia.
3- Patients with lower respiratory tract infection (LRTI).
4- Patients with urinary tract infection (UTI).

Methods
I- Bacteriological study: 1- Samples collection and transport:
A) Blood samples: After palpation of the vein the skin was sterilized in one direction from inside to outside using 2% tincture iodine followed by 70% alc chol. The vein was penetrated using sterile seringe then 5-10ml venous blood were collected under complete aseptic precautions, inoculated into the blood culture bottle (oxoid), containing 80ml of (trypcase 0.05g, L-cystine 0.5g, dextrose 0.5g, yeast extract 0.25g, NaCL 0.05g, and agar 1g. This formula in 9% of distilled waters) then the blood was mixed with the medium, transported to the laboratory and incubated at 37°C (Mermal and Maki, 1993).

B) Wound samples: The wound was exposed and compressed at the edges to ooze bloody discharge then swabbing was done using a sterile swab under complete aseptic precautions. The sample was immersed into a glycerol containing media and transported to the laboratory. (Isenberg et al., 1991)

C) Sputum: The samples were taken from patients with LRTI early in the morning before they use any mouth wash. They were asked to cough deeply and the samples were collected in the sterile, wide necked and leak proof containers and transported immediately to laboratory (Jacobson et al., 1981).

D) Urine: As reported by (Pfaller et al., 1983) : Morning mid stream
urine was collected in sterile, dry, wide necked and leak proof with a tightly fitted lid containers after instruction to cleanse the area around the urethral opening with clean water and soap. In catheterized patients, urine was taken from the catheter under complete aseptic precautions. The first milliliters of urine from the catheters was discarded to wash out any organism that may have lodged in the catheter tip. The samples were transported immediately to the laboratory where they were examined for pus cells and other microscopic characters and culture was done within one hour.

2- Processing of samples: This was done in the microbiology laboratories at the Microbiology Diagnostics and Infection Control Unit (M.D.I.C.U.) along the following lines.

A) Culture:
B) Identification of growth:
Catalase test (Cheesbrough, 1991):

Mannitol fermentation: As reported by (Duguid, 1989).
Coagulase test: Staphytec latex slide agglutination test (Oxoid) (Cheesbrough, 1991).

C- Antimicrobial susceptibility testing: It was done using disk diffusion method (Bauer et al., 1966). A group of antibiotics was used according to the protocol of M.D.I.C.U.

The antibiotic disks used were:
First line antibiotic disks.
Penicillin, Ampicillin, Trimethoprim, Cloxacillin, Gentamicin, Erythromycin, Cefuroxime, Cephradin, Tetracycline.

The strains resistant to the first line were tested against second line:
Second line of antibiotic disks:
Methicillin, Rifampicin, Augmentin, Imipenum, Ciprofloxacin, Vancomycin, Resistance to methicillin was determined by a disk diffusion method on Mueller - Hinton - agar, supplemented with NaCl 2% incubated for 48 hours at 30°C. Resistance was defined as a zone of inhibition of growth around the disk (22mm). (Schmitz et al., 1998).

The strains resistant to methicillin were selected and subjected to genotyping.

II- Genotyping:
Method:
A- Chromosomal DNA extraction (Aushbel et al., 1990):

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1. Bacterial cultures were inoculated in 5ml nutrient broth and incubated over night at 37°C.

2. 1.5ml of the culture was spunne.d for 2 minutes in a microcentrifuge at 10000 rpm until a compact pellet was formed then the supernatant was discarded.

3. Pellet was resuspended in 567ul TE buffer by repeated pipetting.

4. 30ul volume of 10% S.D.S. and 3ul of 20mg/ml proteinase K was added, mixed thoroughly and incubated for 1 hour at 37°C. The solution became viscous as the detergent Lysed the bacterial cell walls, and so there was no need to predigest the bacterial cell wall with lysozyme.

5. 100ul of 5M NaCl was added and mixed thoroughly. The aim of this step was to remove cell wall debris, denatured protein, and polysaccharides complexed to CTAB, while retaining the nucleic acids in solution since a CTAB nucleic acid precipitate will form if salt concentration drops below about 0.5M at room temperature.

6. 80ul of CTAB/NaCl solution was added, mixed thoroughly and incubated for 10 minutes at 65°C.

7. An approximately equal volume (0.7 to 0.8ul) of chloroform/isoamyl alcohol was mixed thoroughly and spunne.d 5 minutes in a microcentrifuge at 10000 rpm.

8. The viscous supernatant was removed to a fresh microcentrifuge tube, leaving the interface behind.

9. An equal volume of phenol/chloroform/isoamyl alcohol, was added to supernatant and spunne.d in a microcentrifuge at 10000 rpm for 5 minutes.

10. The supernatant was transferred to a fresh tube, 0.6 volume of 100% isopropanol was added to precipitate the nucleic acids. The tube was shaken back and forth until a stringy white DNA precipitate became clearly visible. The precipitate was pelleted by spinning briefly at room temperature then was transferred to a fresh tube containing 70% ethanol.

11. The DNA was washed with 70% ethanol to remove residual CTAB and respinne.d 5 minutes at 10000 rpm at room temperature to repellet it. The supernatant was carefully removed and the pellet was left to dry.

12. The pellet was redissolved in 100ul TE buffer.

B- Digestion of chromosomal DNA:

1. In a sterile tube these constituents...
were added in this order: Sterile, deionized water 16.3 ul. RE 10 X buffer 2 ul. Acetylated BSA 0.2 ul. DNA 1 ug/ul 1 ul.

DNA was estimated by spectrophotometer. An U.V absorbance reading of 1 measured at 260 nm indicates an approximate concentration of 50ug/ml of double stranded DNA (Karen et al., 1991).

These constituents were mixed by pipetting then restriction enzyme BgII (0.5ul) was added to reach a final volume of 20 ul.

2- They were mixed gently by pipetting, the tube was closed and centrifuged for few seconds then incubated for 1-4 hours.
3- 5 ul of 6X loading buffer were added and the resulting fragments were separated by electrophoresis.
4- For electrophoretic analysis a 0.9% agarose gel (Promega) was made in 1X TBE buffer and ethedium bromide at a concentration of 0.5 ug/ml was added to it for DNA staining (Karen et al., 1991).
5- Electrophoresis was done for 1.5 hour at 100 volt. Lambda DNA-Hind III digest showing 8 fragments ranging in size from 125 bp to 23.13kb. (Promega) served as molecular size standard (Jordens and Hall, 1988)

RESULTS

Table (1) : Shows nosocomial infection rate: The total number of discharge during the period of study (starting from October 1998 till the end of May 1999) was 36700, the number of nosocomially infected cases was 1296 and so, nosocomial infection rate during the period of study was 1296/36700 = 3.6%

Table (2) : Shows types of nosocomial infections in MUH. Postoperative wound infection (POWI) represented the commonest nosocomial infection during the period of study (47.2%) followed by UTI (22.6%), bacteremia (19.2%) and lastly LRTI which represented (11%)

Table (3) : Shows prevalence and distribution of pathogens in different types of nosocomial infections. Pseudomonas aeruginosa was the commonest pathogen in POWI (20.5%) followed by Staph. aureus (17.3%). Staph. aureus was the commonest pathogen in nosocomial bacteremia (26.9%) followed by CONS (26.1%).

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Klebsiella pneumoniae and E. coli were the commonest pathogens in LRTI and UTI and represented (18.3%) and (29%) respectively. The frequency of different pathogens from nosocomial POWI, bacteremia, LRTI and UTI was illustrated in figures 1, 2, 3 and 4 respectively.

Table (4): Distribution of different nosocomial infections according to the wards. Internal medicine 27.2%, General surgery 20%, and neurosurgery 5%

Table (5): Shows the prevalence of Staphylococcal species in relation to each other. CONS represented (47.2%), (49.2%), (28.1%) and (46.8%) of POWI, bacteremia, LRTI and UTI respectively. MRSA represented (8%), (5.3%), (21.9%) and (8.3%) of POWI, bacteremia, LRTI and UTI respectively. MSSA represented (44.8%), (45.5%), (50%), and (44.9%) of POWI, bacteremia, LRTI and UTI respectively.

Table (6): Shows distribution of MRSA according to the type of nosocomial infection. POWI were the most prevalent and represented (45.7%), both LRTI, bacteremia represented (20%) and UTI represented (14.3%).

Table (7): Shows Frequency of MRSA genotypes. MRSA isolates were discriminated into 8 different genotypes. These were given letters from (A to H). Type A and E formed each (20%). Type B represented (17.1%). Type C, D and F each represented (8.6%). Type G constituted (2.9%) and type H represented (14.2%).

Table (8): Correlation between genotypes and antibiotic sensitivity
- Genotype A was sensitive to ciprofloxacin and imipenum.
- Genotype B was sensitive to tetracycline and imipenum.
- Genotype C was sensitive to tetracycline and trimethoprim.
- Genotype D was sensitive to trimethoprim only.
- Genotype E was sensitive to erythromycin, ciprofloxacin and imipenum.
- Genotypes F and G were sensitive to trimethoprim and ciprofloxacin.
- Genotype H was sensitive to erythromycin and imipenum.

All genotypes were sensitive to rifampicin and vancomycin.

Figure (1): shows sensitivity patterns of MRSA isolates to different an-
tibiotic: the least sensativity was to tetracycline (<10%) and the highest sensativity was for vancomycin and rifampicin (100%)

**Photograph (1)**: Mannitol-salt agar showing yellow colonies of Staph. aureus fermenting mannitol.

**Photograph (2)**: Sensitivity pattern of MRSA isolates to the first line antibiotics.

**Photograph (3)**: Sensitivity patterns of MRSA isolates to the second line antibiotics.

**Photograph (4)**: Resistance of MRSA to the methicillin

**Photograph (5)**: Agarose gel electrophoresis of MRSA genotypes

Lanes from (1) to (8) is from left to right.

-Lane (1) shows: Hind III digest of lambda DNA.

-Lane (2) shows: Genotype F with DNA smearing and one missed band above (9.4)kb.

-Lanes (3 and 6) show: Genotype G with four bands, three ranging from (23.1)kb and (9.4)kb and one opposite (6.5)kb.

-Lane (4) shows: Genotype A.

-Lane (5) shows: Genotype D.

Lane (7) shows: Genotype H with one band opposite (23.1)kb and DNA smearing below (2.3)kb.

-Lane (8) shows: Genotype E with one band opposite (23.1)kb and two bands ranging from (9.4)kb to (4.3)kb.
Table (1): Nosocomial infection rate.

<table>
<thead>
<tr>
<th>NCI</th>
<th>Total discharge</th>
<th>NCIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1296</td>
<td>36700</td>
<td>3.6%</td>
</tr>
</tbody>
</table>

Table (2): Different types of nosocomial infections in MUH.

<table>
<thead>
<tr>
<th>Type of nosocomial infection</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>POWI</td>
<td>612</td>
</tr>
<tr>
<td>Bacteremia.</td>
<td>249</td>
</tr>
<tr>
<td>LRTI.</td>
<td>142</td>
</tr>
<tr>
<td>UTI.</td>
<td>293</td>
</tr>
<tr>
<td>Total</td>
<td>1296</td>
</tr>
</tbody>
</table>
Table (3): Prevalence of pathogens in different types of nosocomial infections.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>POWI No</th>
<th>%</th>
<th>Bacteremia No</th>
<th>%</th>
<th>L.R.T.I No</th>
<th>%</th>
<th>U.T.I No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph. Aureus</td>
<td>106</td>
<td>17.3%</td>
<td>67</td>
<td>26.9%</td>
<td>23</td>
<td>16.2%</td>
<td>27</td>
<td>9.2%</td>
</tr>
<tr>
<td>CONS</td>
<td>95</td>
<td>15.5%</td>
<td>65</td>
<td>26.1%</td>
<td>9</td>
<td>6.3%</td>
<td>27</td>
<td>9.2%</td>
</tr>
<tr>
<td>Strept. Pneumoniae</td>
<td>1</td>
<td>0.16%</td>
<td>1</td>
<td>0.4%</td>
<td>5</td>
<td>3.5%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Strept. Pyogenes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>6.3%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Enterococci</td>
<td>21</td>
<td>3.4%</td>
<td>13</td>
<td>5.2%</td>
<td>14</td>
<td>9.9%</td>
<td>7</td>
<td>2.4%</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>88</td>
<td>14.4%</td>
<td>39</td>
<td>15.7%</td>
<td>26</td>
<td>18.3%</td>
<td>46</td>
<td>15.8%</td>
</tr>
<tr>
<td>E. coli</td>
<td>93</td>
<td>15.2%</td>
<td>35</td>
<td>14.1%</td>
<td>14</td>
<td>9.9%</td>
<td>85</td>
<td>29%</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>125</td>
<td>20.5%</td>
<td>17</td>
<td>6.8%</td>
<td>17</td>
<td>12%</td>
<td>42</td>
<td>14.3%</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>40</td>
<td>6.6%</td>
<td>6</td>
<td>2.4%</td>
<td>1</td>
<td>0.7%</td>
<td>10</td>
<td>3.4%</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>26</td>
<td>4.2%</td>
<td>3</td>
<td>1.2%</td>
<td>4</td>
<td>2.8%</td>
<td>9</td>
<td>3%</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>2</td>
<td>0.32%</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>3.5%</td>
<td>4</td>
<td>1.4%</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>2</td>
<td>0.32%</td>
<td>1</td>
<td>0.4%</td>
<td>1</td>
<td>0.7%</td>
<td>9</td>
<td>3%</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>13</td>
<td>2.1%</td>
<td>2</td>
<td>0.8%</td>
<td>14</td>
<td>9.9%</td>
<td>27</td>
<td>9.3%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>612</td>
<td>100%</td>
<td>249</td>
<td>100%</td>
<td>142</td>
<td>100%</td>
<td>293</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table (4): Distribution of different nosocomial infections according to the wards.

<table>
<thead>
<tr>
<th>Ward</th>
<th>Nosocomial infected cases No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>General surgery</td>
<td>259</td>
<td>20%</td>
</tr>
<tr>
<td>Cardiothoracic surgery</td>
<td>74</td>
<td>5.7%</td>
</tr>
<tr>
<td>Orthopedic surgery</td>
<td>106</td>
<td>8.2%</td>
</tr>
<tr>
<td>Neuro surgery</td>
<td>65</td>
<td>5%</td>
</tr>
<tr>
<td>E.N.T.</td>
<td>78</td>
<td>6%</td>
</tr>
<tr>
<td>Burn unit</td>
<td>135</td>
<td>10.4%</td>
</tr>
<tr>
<td>Internal medicine</td>
<td>352</td>
<td>27.2%</td>
</tr>
<tr>
<td>Pediatrics</td>
<td>227</td>
<td>17.5%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1296</td>
<td>100%</td>
</tr>
</tbody>
</table>

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**Table (5)**: Prevalence of Staphylococcal species isolated from different nosocomial infections in relation to each other.

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>Total No.</th>
<th>CONS</th>
<th>Staph. Aureus</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>%</td>
<td>MSSA</td>
</tr>
<tr>
<td>POWI</td>
<td>201</td>
<td>95</td>
<td>47.2%</td>
<td>90</td>
</tr>
<tr>
<td>Bacteremia</td>
<td>132</td>
<td>65</td>
<td>49.2%</td>
<td>60</td>
</tr>
<tr>
<td>LRTI</td>
<td>32</td>
<td>9</td>
<td>28.1%</td>
<td>16</td>
</tr>
<tr>
<td>UTI</td>
<td>54</td>
<td>27</td>
<td>46.8%</td>
<td>22</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>419</strong></td>
<td><strong>196</strong></td>
<td><strong>46.7%</strong></td>
<td><strong>188</strong></td>
</tr>
</tbody>
</table>

**Table (6)**: Distribution of MRSA isolates according to the type of nosocomial infection.

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>MRSA isolates</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>POWI</td>
<td>16</td>
<td>45.7%</td>
</tr>
<tr>
<td>Bacteremia</td>
<td>7</td>
<td>20.0%</td>
</tr>
<tr>
<td>UTI</td>
<td>5</td>
<td>14.3%</td>
</tr>
<tr>
<td>LRTI</td>
<td>7</td>
<td>20.0%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>35</strong></td>
<td><strong>100.0%</strong></td>
</tr>
</tbody>
</table>
Table (7): Frequency of MRSA genotypes.

<table>
<thead>
<tr>
<th>Pattern of digestion</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>20%</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>17.1%</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>8.6%</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>8.6%</td>
</tr>
<tr>
<td>E</td>
<td>7</td>
<td>20%</td>
</tr>
<tr>
<td>F</td>
<td>3</td>
<td>8.6%</td>
</tr>
<tr>
<td>G</td>
<td>1</td>
<td>2.9%</td>
</tr>
<tr>
<td>H</td>
<td>5</td>
<td>14.2%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>35</td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

List of abbreviations

- Coagulase negative Staph.
- Cons
- Epidemic MRSA.
- EMRSA
- Immunoglobulin G.
- IgG
- Intensive care units.
- ICUs
- Intravenous.
- IV
- Kilo base.
- Kb
- Lower respiratory tract infection.
- LRTI
- Mansoura University Hospital.
- MUH
- Methicillin resistant Staphylococcus aureus.
- MRSA
- Methicillin sensitive Staphylococcus aureus.
- MSSA
- Microbiology Diagnostic and Infection Control Unit.
- MDICU
- Multilocus enzyme electrophoresis.
- MLEE
- Nosocomial infection rate.
- NCIR
- Nosocomial infection.
- NCI
- Polymerase chain reaction.
- PCR
- Postoperative wound infection.
- POWI
- Pulsed field gel electrophoresis.
- PEGE
- Random amplification of polymorphic DNA.
- RAPD
- Restriction endonuclease.
- RE
- Restriction fragment length polymorphisms.
- RFLPs
- Sodium dodecyl sulfate.
- SDS
- Urinary tract infection.
- UTI
Fig (1) : Sensitivity pattern of MRSA to different antibiotics

Photograph (1) : Mannitol-salt agar showing yellow colonies of Staph. aureus fermenting mannitol.

Photograph (2) : Sensitivity pattern of MRSA isolates to the first line antibiotics.

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Photograph (3): Sensitivity pattern of MRSA isolates to the second line antibiotics.

Photograph (4): Resistance of MRSA to the methicillin.

Photograph (5): Agarose gel electrophoresis of MRSA genotypes.

Photograph (6): Agarose gel electrophoresis of MRSA genotypes.
DISCUSSION

In this study, Staphylococci represented (32.8%) of POWI. These were divided into (15.5%) CONS and (17.3%) Staph. aureus including MRSA cases. This finding is consistent with Schaberg et al (1991) who found that CONS represented (13%) and Staph. aureus represented (17%) of surgical wound infections.

In our results, Staphylococci represented (53.0%) of bacteremia (26.1%) were CONS and (26.9%) were Staph. aureus including MRSA. This result correlates with McGown (1985) who reported that the most common etiologic agents of bacteremia are CONS, Staph. aureus, Escherichia coli, Pseudomonas aeruginosa and Enterococci. Also, Archer and Climo (1994) reported that during the 1980s, the incidence of blood stream infection due to CONS increased to the point that they caused more than (25%) of nosocomial blood stream infection.

Similarly, Rello et al (1994) reported that the commonest isolates from cases of nosocomial bacteremia were CONS, Staph. aureus, Pseudomonas aeruginosa and E. coli. Intravascular catheters were the most frequent source of infection. Moreover, Frebourg et al (1999) stated that CONS are the most frequently reported pathogen in nosocomial blood stream infection.

In the present study Staphylococci constituted (22.5%) of which (6.3%) were CONS and (16.2%) were Staph. aureus and Klebsiella pneumoniae represented (18.3%) of LRTI cases. In agreement with our results Gouin et al (1993) reported that the causative pathogens of LRTI were representative of those usually isolated in ICUS: Staph. aureus (19%), Pseudomonas aeruginosa (14%), Klebsiella, Enterobacter and Serratia species (17%) also, Richards et al (1999) found that Pseudomonas aeruginosa (21%) and Staph. aureus (20%) were the most commonly reported isolates in nosocomial pneumonia.

Also, Large et al (1989) reported that the number of nosocomial infections caused by Gram positive organisms is increasing, with the emergence of CONS as important nosocomial pathogen. Furthermore, Armes and Gemmell (1997) reported an increase in Gram positive infections from (30%) to (39.7%). Enterococci increased from (4%) to (7%)
and CONS from (3%) to (7.7%) but Staph. aureus decreased from (17.6%) to (14.7%).

In the present study, CONS represented (47.2%) of Staphylococcal POWI whereas Staph. aureus represented (52.8%) including MRSA which formed (8%). This results correlates with Platt et al (1998) who stated that the principal etiologic agents of surgical wound infection vary with the hospitalís flora and the procedure, with Staph. aureus being a leading pathogen in clean surgery, but CONS are increasingly important in implantation of foreign bodies and device. Also, Edmiston et al (1989) had observed that the majority of prosthetic vascular graft infections are now caused by mucin-producing strains of Staph. epidermidis.

Our results showed that CONS was responsible for (46.8%) of UTI and Staph. aureus was responsible for (53.2%) and by this results we agree with both Demuth et al (1979) who observed that Staph. aureus bacteriuria often indicates metastatic infection of the kidney following bacteremia and Latham et al (1983) who stated that studies have clearly demonstrated pathogenic role of Staph. saprophyticus in urinary tract infections and the vast majority of Staph. saprophyticus infections occur in young women, most commonly in the spring and summer.

In the present work MRSA represented (2.6%) of POWI and (2.8%) of bacteremia. This result correlates with Speller et al (1997) who revealed that in U.K, MRSA bacteremias remained static at about (1.5%) in the period between 1989-1991 and disagrees with Voss and Doebbeling (1995) who reported that MRSA accounted for (28%) of surgical wound infections.

Our results showed that MRSA constituted (2.7%) of the total nosocomial infection. This result agrees with both Voss et al (1994) who stated that the proportion of MRSA ranged from < 1% in Scandinavia to > 30% in Spain and Papia et al (1999) who reported that in the Netherlands, Scandinavia and some parts of U.K. incidence rates of MRSA remained below (5%) and even less than a (0.5%) in the Netherlands.

In the present study, POWI was the commonest nosocomial MRSA infection (45.7%) followed by LRTI, bacteremia (20%) and lastly UTI.

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In the present study, 8 different genotypes of MRSA isolates were recognized. These were A and represented (20%), B (17.1%), C (8.6%), D (8.6%), E (20%), F (8.6%), G (2.9%) and H which represented (14.2%).

In medical patients genotypes F and G were absent while, genotype E was the most prevalent and represented (40%) whereas genotypes C and D were the least prevalent and represented (6.7%). Genotypes A and B represented (13.3%) and genotype H (20%).

In respect to surgical patients, all 8 genotypes were present with genotype A most prevalent (25%) and genotype, E and G least prevalent (5%). Genotypes C, D and H each represented (10%), Genotypes B represented (20%) and F represented (15%).

Cookson et al (1996) stated that until genotypic methods could be standardized it would be difficult to agree the criteria for defining different strains. Clearly each point mutation in a strain could result in up to three band differences and differences may be detectable following DNA inversion or the deletion or insertion of phage,
plasmids or transposons.

SUMMARY AND CONCLUSION

In the present work, NCIR represented (3.6%). The most common NCI was POWI (47.2%) followed by UTI (22.6%), bacteremia (19.2%) and lastly LRTI (11%).

In this study Pseudomonas aeruginosa was the commonest pathogen in POWI (20.5%) and Staph. aureus was the commonest pathogen in nosocomial bacteremia (20.9%). Klebsiella pneumoniae and E. coli were the commonest pathogens in LRTI and UTI and represented (18.3%) and (29%) respective.

In the present study, the commonest Staphylococcal nosocomial infections were bacteremia (53%), followed by POWI (32.8%), LRTI (22.5%) and lastly UTI (18.4%).

Nosocomial MRSA infection represented (2.7%) of the total nosocomial infections and were more prevalent in surgical patients than in medical patients.

The most frequent type of MRSA infection was POWI (45.7%) followed by LRTI infection and bacteremia, each of them were (20%) and lastly UTI (14.3%).

In surgical patients infected with MRSA, vancomycin and rifampicin were most effective (100%) followed by imipenem (75%), trimethoprim (30%), ciprofloxacin (25%), tetracycline (10%) and lastly erythromycin (5%).

MRSA isolates were divided into 8 groups depending on the pattern of digestion with the restriction enzyme Bgl II. Type A was the most prevalent (20%) and type G was the least prevalent (2.9%). In medical patients, types F and G were absent and type E was the most frequently isolated. In surgical patients, all 8 genotypes were present with type A most frequently isolated in these patients.

Analysis of MRSA isolates from various hospital outbreaks by genotypic fingerprinting techniques can be used to identify transmission routes and reservoirs of MRSA clones in the hospital environment.

We recommend construction of Microbiology Diagnostic and Infection Control Units (MDICU) in all Hospitals with intimate cooperation with clinical...
departments specially surgical wards with generalization of gonotybing techniques for prophylactic measures against infection and hence: limitation of both morbidity and mortality.

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عدوى المستشفيات مع إشارة خاصة لدراسة التصنيف الوراثي للميكروب الوراثي للميكروب العنقودي الذهبي المكتسب المقاوم للمزئينين: تقنية عالية الدقة ودلالات متخصصة لمساحة مصادر وطرق العدوى

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قسم الميكروبيولوجيا والمناعة الطبية ووحدة التشخيص العملي
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عندما يحدث للمرضى الذين أدخلوا المستشفى بدون أعراض العدوى وظهرت الأعراض بعد مدة لا تقل عن 72 ساعة، تعتبر هذه العدوى من الأهمية بمكان حيث أنها تكون مصحوبة بمعدلات عالية في الإعتلا والوفيات.

وكان الهدف: دراسة مدى انتشار هذه العدوى والميكروبات والحساسية لها وحساسية هذه الميكروبات للمضادات الحيوية مع إشارة خاصة لدراسة التصنيف الوراثي للميكروب العنقودي الذهبي المكتسب المقاوم للمزئينين باستخدام الأزمتات القاطعة للحامض النووي.


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الطرق المستخدمة: تم إخضاع العينات المأخوذة من جميع المرضى للدراسات البكترية (مزرعة التعرف على الميكروبات - اختبارات الحساسية للمضادات الحيوية) وبالنسبة للميكروبات العنقوديّة، النسيجي، الدهني للميزوسبيلين فقط تم عمل تصنيف وراثي لاستخدام الأدوية القاطعة للحمض النووي.

النتائج: كان معدل إنتشار العدوى المكتسبة (2/3) وكان أكثر الالتهابات إنتشاراً التهابات الجروح، التهابات المجرى البول، العدوى المكتسبة في الدم ثم إنتشار الجهاز التنفيسي السفلي وكان ميكروبات السوودوموناس (20/27)، الميكروبات العنقوديّة الدهني (16/27)، عضويات الكليوبسيلا (18/27) وعصويات القولون (29/27) الميكروبات الأكثر شيوعاً في رئياسات الجروح، العدوى الدم، التهابات الجهاز التنفيسي السفلي والتغطية الجهازية الباردة وذلك بالتنباع: الميكروبات العنقوديّة الدهني المقارن للميزوسبيلين يسب عودة (20/27) من العدوى المكتسبة وكان أكثر شيوعاً في مرضى أقسام الجراحة من مرضى أقسام الباطنة. الفانكوسيبسين والريفاميسينين هم أكثر مضادات الحيوية فعالية في علاج هذا الميكروبات في مرضى الجراحة. استطاعت الدراسة الحالية أن تصنف هذه الميكروبات وراثياً إلى (8) مجموعات إعتماداً على طريقة القطع الناتجة عن استخدام الإستيمات القاطعة للحمض النووي، جميع المجموعات أكثر المجموعات إنتشاراً، وكانت نسبتها (20/8) والمجموعة ج كانت أقلها إنتشاراً ونسبتها (9/8). المجموعات جمج مجموعات لم تكن موجودة في مرضى أقسام الباطنة والمجموعة (إ) كانت الأكثر شيوعاً. كل المجموعات كانت مماثلة في مرضى أقسام الجراحة وكانت المجموعة (أ) الأكثر شيوعاً.

الاستنتاجات: العدوى المكتسبة داخل المستشفيات تمثل أهمية خاصة لما لها من درجات عالية من المشاكل والوفيات وينبغي إستخدام الدراسات البكترية لمعرفة نوع الميكروبات السببية لها وكذلك حساسيتها للمضادات الحيوية المناسبة لها. يمكن إستخدام التصنيف الوراثي لهذه الميكروبات لمعرفة طرق إنتشارها ومعرفة حامل العدوى داخل المستشفى.

النصح: تنصب إنشاء وحدات للميكروبيولوجي لتشخيص وعالية العدوى (MDICU) بجميع المستشفيات مع التعاون بينها وبين الأقسام الإكلينيكية وخصوصاً أقسام الجراحة مع تعميم استخدام التصنيف الوراثي للميكروبات وذلك لعمل الإجراءات الوقائية للعدوى ومن ثم تقليل معدلات الاعتقال والوفيات.

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