ORIGINAL ARTICLE

Antibacterial susceptibility of bacteria isolated from burns and wounds of cancer patients

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Abstract In this study 540 burns and wound swabs were collected from cancer patients of some Egyptian hospitals. The single infection was detected from 210, and 70 cases among wounded and burned patients, while mixed infection was 30 and 45, respectively. We recovered where 60 isolates of Pseudomonas aeruginosa, 60 isolates of Staphylococcus aureus, 7 isolates of Staphylococcus epidermidis, 4 isolates of Streptococcus pyogenes, 25 isolates of Escherichia coli, 23 isolates of Klebsiella pneumoniae and 27 isolates of Proteus vulgaris from 355 burn and surgical wound infections. All bacterial isolates showed high resistance to the commonly used β-lactams (amoxycillin, cefaclor, ampicillin, vancomycin, amoxicillin/clavulonic), and low resistance to imipenim and ciprofloxacin. Plasmid analysis of six multidrug resistant and two susceptible bacterial isolates revealed the same plasmid pattern. This indicated that R-factor is not responsible for the resistance phenomenon among the isolated opportunistic bacteria. The effect of ultraviolet radiation on the isolated bacteria was studied.

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1. Introduction

Hospital-acquired infections remain a cause of morbidity, extended hospital stay and death for patients (Holzheimer et al., 1990; Pruitt et al., 1998; Naeem et al., 2006). The burn and wound represent a susceptible site for opportunistic colonization by organisms of endogenous and exogenous origin (Pruitt et al., 1998). Bacterial infections in burn and wound patients are common and are difficult to control. Sepsis consequently is common and sepsis is often fatal (Lee et al., 1990; Armour et al., 2007). In Egypt, nosocomial infection constitutes a major problem. It requires more interest and attention than it currently receives as it is responsible for a great deal of morbidity and mortality among hospitalized patients in addition to unavailability of records, statistics or enough information about the problem as well as lack of universal program or approach to control it (Abdel Rahman et al., 2010). Burns, wounds, trauma, multiorgan failure and use of invasive devices for surgery, and exposure of microorganisms in the environment of hospital to a number of antimicrobial agents leading
to selective resistance are all some of the factors facilitating colonization, transmission and susceptibility to infection (Poh and Yeo, 1993).

The infection of burn wounds with multiple organisms, with superadded problem of drug resistance, illustrates the need for a drug policy by the hospitals for burn patients. The isolated bacteria exhibited multiple resistance to antibiotics (Roberts et al., 2008). Burns provide a suitable site for bacterial multiplication and are more persistent richer sources of infection than surgical wounds, mainly because of the larger area involved and longer duration of patient stay in the hospital (Agnihotri et al., 2004). Bacterial infections in burn and wounds are common and are difficult to control. Sepsis consequently is common and sepsis is often fatal (Lee et al., 1990; Armour et al., 2007).

Plasmids are extrachromosomal self-replicating genetic materials found in a variety of bacterial species and not essential for growth of bacteria. Plasmids could carry genes that code for drug resistance, virulence, production of antimicrobial agents and metabolic activities (Ibrahim, 2002).

The aims of this paper are to isolate and identify bacterial species causing burn and surgical wound post infections from some Egyptian hospitals as well as determination of the antimicrobial susceptibility of the isolated microorganisms and plasmid profile analysis of the most frequent isolated organisms which acquired multiple drug resistance.

2. Materials and methods

2.1. Sample collection

Swabs were collected from 540 cancer inpatients (radiotherapy treated) of burn and surgical units and transported aseptically to bacteriological labs for analysis. The patients' samples comprise 365 post operative wounds and 175 burns from three hospitals namely: El-Hussein University, Ain-Shams University and Mansoura University.

2.2. Media

The following media were prepared according to the instructions of the manufacturer, which include MacConkeys agar, nutrient agar, nutrient broth, mannitol salt agar, tryptcase soya agar and urea agar base. On the other hand, blood agar medium, indole test medium, sugar fermentation medium, gelatin liquefaction medium and motility test medium were prepared according to Collee et al. (1996).

Swabs were taken from all septic wounds, one week after radiotherapy treatment. Swabs were transported into 2 ml tryptcase soya bean broth and incubated aerobically at 37 °C for 18 h. Then, one loopful from each sample was streaked on MacConkey’s agar, mannitol salt agar, blood agar; the plates were incubated at 37 °C for 24-48 h. Bacterial growth was identified by colony characteristics, blood hemolysis, microscopic examination of Gram stained preparations and motility techniques. Biochemical activities including oxidase test; glucose, lactose and mannitol fermentation, indole production, gelatin liquefaction, catalase activity, nitrate reduction, urease production, \( \text{H}_2\text{S} \) production, coagulase and pigment production were performed to confirm the identification of each isolate according to the methods of Manual of Methods for General Bacteriology (1981).

2.3. Antimicrobial susceptibility test

The identified isolates were tested using some antibiotics, such as amoxicillin (25 μg), Cefaclor (25 μg), ampicillin (30 μg), amoxicillin/clavulonic acid (25 μg), Ciprofloxacin (10 μg), Imepenim (10 μg) and Vancomycin (35 μg) (Oxoid, UK). The test was performed according to the Kirby–Bauer technique (Bauer et al., 1966) and results interpreted using chart of NCCLS (1994).

2.4. Plasmid profile

Plasmids of multi-drug resistant isolates were analyzed by rapid screening procedure for plasmid DNA (Kado and Liu, 1981). Plasmid DNA of susceptible antibiotic was used as a control for comparative studies.

2.5. Effect of ultraviolet (UV) irradiation on viability of bacterial growth

Nearly 2 × 10^8 cells/ml, for each isolate, were exposed to UV Lamp at 2600 nm (famed 1, Poland) at a distance of 30 cm for 0, 30, 60, 90, 120 and 150 s, respectively. 0.05 ml was spread (homogeneously) over nutrient agar plates after each exposure in order to obtain the viable cell count. Assay plates were also inoculated, after being diluted to a factor of 2 × 10^5 as 0.03 and 0.07 ml per plate, respectively, prior to exposure, to confirm the viable cell count in the original culture. One percent (1%) survival level of each isolate was calculated from the given results to show the killing effect of UV light. Results were expressed as viable cell count after each exposure as well as the 1% survival level of each isolate under investigation.

3. Results

Out of 540 cancer patients of burn and surgical wounds, 355 cases were infected with bacteria and represent 65.74% of total patients included in this study. From the 260 cases of El-Hussein University Hospital, 180 cases developed infections with an incidence rate of 69.23%. These comprised of 125 wounds infections out of 185 (67.57%) and 55 infections out of 75 (73.33%) burn cases. Out of 150 cases of surgical operations of Ain-Shams University Hospital, 90 (60%) developed wound infections. While out of 85 burn cases from Mansoura University Hospital, 45 (52.94%) developed infections (Table 1).

Table 2 shows single and mixed bacterial infections of cancer patients with wounds and/or burns. The prevalence of single bacterial infections among wounded patients was high (280 cases) and only 75 cases showed mixed bacterial infections. However, the prevalence of mixed bacterial infection in the case of burned patients was significantly higher than surgically wounded patients (42.8%), while single bacterial infections were (57.2%). At El-Hussein University Hospital out of 125 wounded patients only 15 (12%) were having mixed bacterial infections and only 20 (57.14) out of 55 burned patients were having mixed bacterial infection. At Ain-Shams University Hospital, 10 (11.11%) out of 90 wounded patients were infected with mixed infection. However, at Mansoura University Hospital, there were 20 (44.4%) out of 45 burned patients having mixed bacterial infection.
The prevalence of bacterial species isolated from wounded and burned patients: *Pseudomonas aeruginosa* was the most frequent microorganism isolated from burned patients (30 isolates, 36.14%), followed by *Staphylococcus aureus* (20 isolates, 30.12%), *Proteus vulgaris* (15 isolates, 18.07%), *Klebsiella pneumoniae* (8 isolates, 9.64%) and *Escherichia coli* (5 isolates, 6.02%). While *S. aureus* was the most frequent microorganism isolated from wounds, it was isolated from 35 wounded patients (28.23%) followed by *P. aeruginosa* (30 isolates, 24.19%), *E. coli* (20 isolates, 16.13%), *K. pneumoniae* (15 isolates, 12.10%), *P. vulgaris* (12 isolates, 9.68%), *Staphylococcus epidermidis* (7 isolates, 5.65) and *Streptococcus pyogenes* (4 isolates, 3.23%) (Table 3).

The prevalence of bacterial species isolated from cancer patients.

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Burn</th>
<th>Wound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of isolates</td>
<td>%</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>30</td>
<td>36.14</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>25</td>
<td>30.12</td>
</tr>
<tr>
<td><em>P. vulgaris</em></td>
<td>15</td>
<td>18.07</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>5</td>
<td>6.02</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>8</td>
<td>9.64</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total no. of bacterial isolates</td>
<td>83</td>
<td>100</td>
</tr>
</tbody>
</table>

3.1. Antibacterial susceptibility pattern

*K. pneumoniae* isolates were resistant to amoxicillin and amoxicillin/clavulonic acid in a ratio of 90% while 27.2% of the isolates were resistant to ciprofloxacin, 67% to cefaclor, 76% to ampicillin, and 63% to imipenim (Fig. 4). Fig. 5 showed that isolates of *P. vulgaris* were resistant to cefaclor (75%), imipenim and vancomycin, each (72.1%), and ciprofloxacin (25%). The resistance of the *S. aureus* isolates showed that 29% of the isolates were resistant to ciprofloxacin, 64% to amoxicillin, 66.9% to cefaclor and 47% to imipenim (Fig. 6).

Ciprofloxacin was found to be the most powerful antibiotic and only 17% of *E. coli* isolates were resistant. However, *E. coli* isolates were 52%, 23%, and 18% resistant to amoxicillin/clavulonic acid, imipenim and vancomycin, respectively (Fig. 7).

*P. aeruginosa* is known to be naturally resistant to amoxicillin and ampicillin. These antibiotics were tested against *P. aeruginosa* to make a comparison with other organisms possible. All isolates of *P. aeruginosa* were resistant to amoxicillin, cefaclor and amoxicillin/clavulonic acid. Data showed 93.4%, 90% and 80% of the isolates were resistant to vancomycin, ampicillin and Imipenim, respectively (Fig. 8).

3.2. Mutagenic effect of UV irradiation

The experiment of UV mutant was conducted after preliminary tests on seven types of bacteria to determine the suitable
initiation time of irradiation by which the plates were countable. It can be concluded from (Table 4) that the number of mutant was increased as the UV doses increased. \( E. \text{coli} \) and \( S. \text{pyogenes} \) currently not tolerated UV irradiation over 90 s. The most tolerant organism to UV was \( S. \text{epidermidis} \) \((3.7 \times 10^2)\) followed by \( S. \text{aureus} \) \((2 \times 10^2)\) and \( P. \text{vulgaris} \) \((1 \times 10^2)\) CFU/ml.

### 3.3. Plasmid analysis

Eight isolates of \( P. \text{aeruginosa} \) were tested against different antimicrobial agents; two of them were different antibiotic sensitivity patterns. Plasmids of these eight strains were analyzed and results revealed that all the six \( P. \text{aeruginosa} \) isolates contained the same plasmid pattern. On the other hand, \( P. \text{aeruginosa} \) isolates (two isolates) that were sensitive to the above mentioned antibiotic was used as a control and gave the same plasmid pattern.

Fig. 9 shows photograph of plasmid analysis as follows: Pattern I: (two isolates) which were resistant to ciprofloxacin and amoxicillin; lanes 1 and 2. Pattern II: (one isolate) shows resistance to amoxicillin; lane 3. Pattern III: (two isolates), shows resistance to amoxicillin and cefaclor; lanes 4 and 5. Pattern 4: (one isolate), which was resistant to imipenem, ciprofloxacin, and amoxicillin; lane 6. The other two lanes: 7 and 8 represented two sensitive isolates.

### 4. Discussion

Nosocomial infections play a role in quality and control in healthcare. Surveillance of these infections is the only way to gain more insight into their frequency and cause (Beaujean et al., 2002). Surgical site infections are a problem in all fields of surgery (Steinbrecher et al., 1992). In addition, burned patients are at a high risk for nosocomial infections by multiresistant bacteria, a large proportion of which are gram negative (Mokaddas et al., 1998). Within 24 h, burned patients can start suffering from opportunistic bacterial attacks that can vary from simple infections, such as those easily treatable by antibiotics to more complicated bacteria, which may have natural or acquired resistance to drugs. Infection by multiple drug resistant bacteria could create additional complexity to the problem (Ahmad, 2002).

Hussein et al. (1989) stated that the infection at the burn unit was 84.9%. The same result obtained by Mago (2009) who made a burn sepsis revealed that bacterial colonization reached 80.6%; also, Cremer et al. (1996) found the infection in burn unit was 94%. The incidence of bacterial infection of burned patients obtained from El-Hussein University Hospital and Mansoura University Hospital was 73.33% and 52.94%, respectively. Our results obtained agreed with other workers including Hussein et al. (1989), Cremer et al. (1996) and Mago (2009) who stated that the infection at the burn unit was 84.9%, 94% and 80.6%, respectively. Naeem et al. (2006) at the burns centre, totally agreed with the result obtained from Mansoura University Hospital where the incidence of burn infection was 10.1%. The marked reduction in the percentage of infection in Mansoura University Hospital may be attributed to the advanced surgical techniques, instruments and the precise application of aseptic technique.

\( P. \text{aeruginosa} \) was the most common organism encountered in burn infection \((n = 30, 36.14\%)\) as indicated in (Table 3). The obtained results agreed with Cremer et al. (1996) and Branski et al. (2009) who found \( P. \text{aeruginosa} \) in burn infection in a percentage of 49% and 53.9%, respectively. \( P. \text{aeruginosa} \) remains a significant pathogen in burn infection, its pathogenicity being associated with the production of a cocktail of virulence determinants, which is regulated by a population density-dependant mechanism and diffusion of signaling.
molecules in the burn-wound environment (Koeber et al., 2002). The high predominance of *P. aeruginosa* among the burned patients must reflect a proper attention to the wounds of the burned patients.

On other hand, the isolated *P. aeruginosa* were at a lower frequency rate as in Ain-Shams Hospital where *P. aeruginosa* Incidence of 20% of burn exudates (Saleh, 2000). Also, Kluyatmans (2007) isolated *P. aeruginosa* from burn exudates at frequencies of 19.7% and 21%, respectively.

The second most important microorganism isolated from burned patients was *S. aureus* (*n* = 25, 30.12%). Hussein et al. (1989) and Mohamed et al. (2000) also isolated *S. aureus* as the second most important microorganism encountered in burned patients following *P. aeruginosa*, *S. aureus* and *S. pyogenes* that were found to tolerate sunlight up to 120 min of exposure.

These results agreed with Mohamed et al. (2000) who isolated *K. pneumonia* and *E. coli* from burn infections at frequencies of 2% and 7%, respectively in addition to *Prot. mirabilis*.

On the other hand, this result was not in agreement with Khashaba (1981) who found that the most predominant isolated organism was *S. aureus* (53.8%), followed by Klebsiella sp. (26.3%), *P. aeruginosa* (18.8%), Proteus sp. (6.3) and *E. coli* (3.8%). On the other hand, this result was not in agreement with Khashaba (1981) who found that the most predominant isolated organism was *S. aureus* (53.8%), followed by Klebsiella sp. (26.3%), *P. aeruginosa* (18.8%), Proteus sp. (6.3) and *E. coli* (3.8%).

This variation in the frequency rates may be due to variations in both environmental conditions and attitudes toward management of the burn wound. The number of wound infections involved was 365 patients (150 patients from Ain-Shams University Hospital, 30 patients from Mansoura University Hospital, and 185 patients from El-Hussein University Hospital). Out of 30 patients from Mansoura University Hospital, 25 cases (83.33%) developed bacterial infection and out of 185 patients from surgery department at El-Hussein Hospital, 125 patients (67.57%) developed bacterial infections. This result was in accordance with Aganovic et al. (1994) whose rate of nosocomial infection of post-operative wounds was 69.45%.
On the other hand, post-operative infection was at a lower rate. Mostafa (2006) found that surgical wound infection was the commonest nosocomial infection (40%). Mohamed et al. (2000) found that the infection rate in postoperative wounds was 37.3%. Our results disagreed with the results obtained by Holzheimer et al. (1990), Marroni et al. (2003), Lee et al. (1990) and El-Daghstany (1992). They found that the overall nosocomial infection of post-operative was 13%, 2.1%, 11.4%, and 11.1%, respectively, while Nageb (1990), Ellahawy et al. (1992) and Abussaud (1996) found that the overall incidence of surgical infections was 8.7%, 9%, and 8%, respectively.

The most common microorganisms isolated from wounded patients were \( S.\ aureus \) \((n = 35, 28.23\%)\). The obtained result more or less agreed with the result of Mostafa (2006) who found \( S.\ aureus \) in significantly high prevalence in wound infection (36.2%). Also Mohamed et al. (2000) reported that \( S.\ aureus \) was the most common pathogen responsible for the post operative wound infection (33.7%).

Different results were obtained by Nageb (1990) and Zaghoul (1993), they isolated \( S.\ aureus \) at a prevalence of 19.2% and 13.2% respectively. Other isolated \( S.\ aureus \) at higher frequency rates: Khozam (1987) (59%) and Master et al. (2010) (88%).

The second most important microorganism isolated from the wounds was \( P.\ aeruginosa \) \((n = 30, 24.19\%)\). Mohamed et al. (2000) reported that \( P.\ aeruginosa \) was one of the most common pathogens responsible for the post-operative wound infection (25.3%), Saleh (2000) and Cestari et al. (1999) isolated \( P.\ aeruginosa \) from 20% and 31.2% of surgical wound exudates samples, respectively. Low frequency rate was presented by Nageb (1990) and Kluytmans (2007) they isolated \( P.\ aeruginosa \) from 11.5% and 8% of surgical wound exudates, respectively.

Mohamed et al. (2000) isolated \( K.\ pneumonia \) (18%) from the post-operative wound infection. Khozam (1987) found \( Klebsiella \) sp. (9.9%). Abussaud (1996) isolated \( Klebsiella \) sp. at (10%).
The policy of antibiotic treatment was always based on in vitro susceptibility test. *P. aeruginosa* was always among the bacteria most readily acquiring resistance toward antimicrobial drugs. It could cause septicemia in burned patients. So careful attention should be paid to inpatients whose wounds are colonized by this organism (Ashour, 2000).

Seven antimicrobial agents were used which are Amoxycillin, Cefaclor, Imipenim, Amoxicillin/clavulonic acid, Ciprofloxacin, Vancomycin, and Ampicillin.

Amoxycillin, Cefaclor, were of low activity, and this may be attributed to the extensive use of these drugs. The resistance pattern toward Amoxicillin was as follows: *P. aeruginosa* (100%), *E. coli* (89.5%), *K. pneumonia* (90.0%), *P. vulgaris* (80.1%) and *S. aureus* (59%). The resistance pattern to Cefaclor was as follows: *P. aeruginosa* (100%), *E. coli* (79.9%), *K. pneumonia* (67%), *Prot. mirabilis* (69%), and *S. aureus* (61%). The resistance pattern to Imipenim was the most potent antimicrobial agents against the isolated gram-negative bacilli where 89% of *P. aeruginosa*, 82% of *E. coli*, 100% of *K. pneumonia* and 97.5% of *P. vulgaris* isolates were resistant to imipenim. Ciprofloxacin comes after imipenim in activity against the isolated Gram-negative bacilli.

Imipenim was the most active antibiotic against *P. aeruginosa*, El-Naggar (1984), Mohamed et al. (2000) and Saleh (2000), they demonstrated the resistance of *P. aeruginosa* isolates to imipenim were 98%, 85% and 100%, respectively. Survey at the North of Portugal was performed to assess the level of susceptibility to the most common antibiotics with

![Figure 9](image_url)

Photograph of ethidium bromide gel showing marker in left hand side followed by *Pseudomonus aeruginosa* which was resistant to ciprofloxacin and amoxicillin; lanes 1 and 2, *Pseudomonas aeruginosa* which was resistant to amoxicillin; lane 3, *Pseudomonas aeruginosa* which was resistant to amoxicillin and cefaclor; lanes 4 and 5, *Pseudomonas aeruginosa* which was resistant to imipenim, ciprofloxacin, and amoxicillin, lane 6 and sensitive *Pseudomonas aeruginosa*, lanes 7 and 8.
anti-pseudomonal activity against \textit{P. aeruginosa}. It revealed that out of 525, 10\% of the isolates were resistant to imipenim (Gardoso et al., 2002). Saleh (2000) found that 64\% of \textit{P. aeruginosa} strains were resistant to ciprofloxacin. El-Daker (2002) found that imipenim and ciprofloxacin were the most effective antibiotics toward multidrug resistant Gram-negative bacilli at Ain-Shams University Hospitals. Hussein et al. (2001) investigated that the sensitivity rate for imipenim was 71\% and for ciprofloxacin 52\%, in contrast most of the tested isolates showed resistance to the third generation Amoxycillin/ clavulonic acid (90\%).

The prevalence of Cefaclor-resistant \textit{P. aeruginosa} in Thailand was 24\%, which is higher than the value reported for \textit{P. aeruginosa} isolated of North America and Europe (Girlich et al., 2002). Vancomycin was the effective drug in surgical and medical patients infected with methicillin-resistant \textit{S. aureus} isolated from Ain-Shams University Hospitals. Susceptibility testing of isolates \textit{S. aureus} collected from different hospitals in Riyadh, Saudi Arabia to vancomycin showed that all isolates were sensitive (Fouda et al., 2005).

The effect of UV radiation on isolated bacteria was carried out to figure out the role of UV to control the growth of the bacteria contaminating the burns and wounds. The lethal effect of UV on the isolated bacteria varied considerably among the species examined, e.g., it is as low as 90 s in the case of \textit{E. coli} and \textit{S. pyogenes}, whereas it is as high as 150 s in case of \textit{S. aureus}, \textit{P. vulgaris} and \textit{S. epidermidis}. Bacteria that were resistant to UV rays and endured their effect for up to 150 s may be characterized by a DNA with a high GC mol.\% value and consequently, low thymine content, thus reducing the probability for thymine dimerization. Bacteria may also exhibit high activity and complete repair systems for photo reaction, excision repair and post-replication repair.

In the present work six multi-drug resistant and two sensitive strains of \textit{P. aeruginosa} were selected for study concerning their plasmid profile. They were tested against seven antimicrobial agents. The \textit{P. aeruginosa} isolates revealed five different antibiotic patterns. The result revealed that all the eight \textit{P. aeruginosa} isolates contained the same plasmid pattern, which means that there was no correlation between plasmid pattern and their antimicrobial activity. This result was in agreement with that of Fouda et al. (2005).

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