Antibacterial susceptibility of bacteria isolated biology essay

Science, Biology

from burns and wounds of cancer patientsAbstract In this study 540 burns and wound swabs were collected from cancer patients of someEgyptian hospitals. The single infection was detected from 210, and 70 cases among woundedand burned patients, while mixed infection was 30 and 45, respectively. We recovered where 60 isolatesof Pseudomonas aeruginosa, 60 isolates of Staphylococcus aureus, 7 isolates of Staphylococcusepidermidis, 4 isolates of Streptococcus pyogenes, 25 isolates of Escherichia coli, 23 isolates ofKlebsiella pneumoniae and 27 isolates of Proteus vulgaris from 355 burn and surgical wound infections. All bacterial isolates showed high resistance to the commonly used b-lactams (amoxycillin, cefaclor, ampicillin, vancomycin, amoxicillin/clavulonic), and low resistance to imepenim andciprofloxacin. Plasmid analysis of six multidrug resistant and two susceptible bacterial isolatesrevealed the same plasmid pattern. This indicated that R-factor is not responsible for the resistancephenomenon among the isolated opportunistic bacteria. The effect of ultraviolet radiation on theisolated bacteria was studied. 1. IntroductionHospital-acquired infections remain a cause of morbidity, extended hospital stay and death for patients (Holzheimeret al., 1990; Pruitt et al., 1998; Naeem et al., 2006). The burnand wound represent a susceptible site for opportunistic colonizationby organisms of endogenous and exogenous origin(Pruitt et al., 1998). Bacterial infections in burn and woundpatients are common and are difficult to control. Sepsis consequentlyis common and sepsis is often fatal (Lee et al., 1990; Armour et al., 2007). In Egypt, nosocomial infection constitutesa major problem. It requires more interest and attentionthan it currently receives as it is responsible for a great deal ofmorbidity and mortality among

hospitalized patients in additionto unavailability of records, statistics or enough informationabout the problem as well as lack of universal program orapproach to control it (Abdel Rahman et al., 2010). Burns, wounds, trauma, multiorgan failure and use of invasive devicesfor surgery, and exposure of microorganisms in the environmentof hospital to a number of antimicrobial agents leadingto selective resistance are all some of the factors facilitatingcolonization, transmission and susceptibility to infection(Poh and Yeo, 1993). The infection of burn wounds with multiple organisms, withsuperadded problem of drug resistance, illustrates the need for adrug policy by the hospitals for burn patients. The isolated bacteriaexhibited multiple resistance to antibiotics (Roberts et al., 2008). Burns provide a suitable site for bacterial multiplicationand are more persistent richer sources of infection than surgicalwounds, mainly because of the larger area involved and longerduration of patient stay in the hospital (Agnihotri et al., 2004). Bacterial infections in burn and wound patients are commonand are difficult to control. Sepsis consequently is commonand sepsis is often fatal (Lee et al., 1990; Armour et al., 2007). Plasmids are extrachromosomal self-replicating geneticmaterials found in a variety of bacterial species and not essentialfor growth of bacteria. Plasmids could carry genes thatcode for drug resistance, virulence, production of antimicrobialagents and metabolic activities (Ibrahim, 2002). The aims of this paper are to isolate and identify bacterialspecies causing burn and surgical wound post infections fromsome Egyptian hospitals as well as determination of the antimicrobialsusceptibility of the isolated microorganisms andplasmid profile analysis of the most frequent isolated organismswhich acquired multiple drug resistance. 2.

Materials and methods2. 1. Sample collectionSwabs were collected from 540 cancer inpatients (radiotherapytreated) of burn and surgical units and transported aseptically to bacteriological labs for analysis. The patients' samples comprise365 post operative wounds and 175 burns from three hospitalsnamely: El-Hussein University, Ain-Shams Universityand Mansoura University. 2. 2. MediaThe following media were prepared according to the instructionsof the manufacturer, which include MacConkeys agar, nutrient agar, nutrient broth, mannitol salt agar, trypticasesoya agar and urea agar base. On the other hand, blood agarmedium, indole test medium, sugar fermentation medium, gelatinliquefaction medium and motility test medium were preparedaccording to Collee et al. (1996). Swabs were taken from all septic wounds, one week afterradiotherapy treatment. Swabs were transported into 2 ml trypticasesoya bean broth and incubated aerobically at 37 _C for18 h. Then, one loopful from each sample was streaked onMacConkey's agar, mannitol salt agar, blood agar; the plateswere incubated at 37 _C for 24–48 h. Bacterial growth wasidentified by colony characteristics, blood hemolysis, microscopicexamination of Gram stained preparations and motilitytechniques. Biochemical activities including oxidase test; glucose, lactose and mannitol fermentation, indole production, gelatin liquefaction, catalase activity, nitrate reduction, ureaseproduction, H2S production, coagulase and pigment productionwere performed to confirm the identification of eachisolate according to the methods of Manual of Methods forGeneral Bacteriology (1981). 2. 3. Antimicrobial susceptibility testThe identified isolates were tested using some antibiotics, suchas amoxicillin (25 lg), Cefaclor (25 lg), ampicillin (30 lg), amoxicillin/clavulonic acid (25 lg),

Ciprofloxacin (10 lg), Imepenim (10 lg) and Vancomycin (35 lg) (Oxoid, UK). Thetest was performed according to the Kirby–Bauer technique(Bauer et al., 1966) and results interpreted using chart ofNCCLS (1994). 2. 4. Plasmid profilePlasmids of multi-drug resistant isolates were analyzed by rapidscreening procedure for plasmid DNA (Kado and Lui, 1981). Plasmid DNA of susceptible antibiotic was used as acontrol for comparative studies. 2. 5. Effect of ultraviolet (UV) irradiation on viability ofbacterial growthNearly 2 · 108 cells/ml, for each isolate, were exposed to UVLamp at 2600 nm (famed 1, Poland) at a distance of 30 cmfor 0, 30, 60, 90, 120 and 150 s, respectively. 0. 05 ml wasspread (homogeneously) over nutrient agar plates after eachexposure in order to obtain the viable cell count. Assay plateswere also inoculated, after being diluted to a factor of 2 · 105as 0. 03 and 0. 07 ml per plate, respectively, prior to exposure, to confirm the viable cell count in the original culture. Onepercent (1%) survival level of each isolate was calculatedfrom the given results to show the killing effect of UV light. Results were expressed as viable cell count after each exposureas well as the 1% survival level of each isolate underinvestigation. 3. ResultsOut of 540 cancer patients of burn and surgical wounds, 355cases were infected with bacteria and represent 65. 74% of totalpatients included in this study. From the 260 cases of El-HusseinUniversity Hospital, 180 cases developed infections with anincidence rate of 69. 23%. These comprised of 125 woundsinfections out of 185 (67. 57%) and 55 infections out of 75(73. 33%) burn cases. Out of 150 cases of surgical operationsof Ain-Shams University Hospital, 90 (60%) developed woundinfections. While out of 85 burn cases from Mansoura UniversityHospital, 45 (52. 94%) developed infections (Table 1). Table 2

shows single and mixed bacterial infections of cancerpatients with wounds and/or burns. The prevalence of singlebacterial infections among wounded patients was high (280cases) and only 75 cases showed mixed bacterial infections. However, the prevalence of mixed bacterial infection in thecase of burned patients was significantly higher than surgicallywounded patients (42. 8%), while single bacterial infectionswere (57. 2%). At El-Hussein University Hospital out of 125wounded patients only 15 (12%) were having mixed bacterialinfections and only 20 (57. 14) out of 55 burned patients werehaving mixed bacterial infection. At Ain-Shams UniversityHospital, 10 (11. 11%) out of 90 wounded patients were infectedwith mixed infection. However, at Mansoura UniversityHospital, there were 20 (44. 44%) out of 45 burned patientshaving mixed bacterial infection. The prevalence of bacterial species isolated from woundedand burned patients: Pseudomonas aeruginosa was the mostfrequent microorganism isolated from burned patients (30isolates, 36. 14%), followed by Staphylococcus aureus (20 isolates, 30. 12%), Proteus vulgaris (15 isolates, 18. 07%), Klebsiellapneumonea (8 isolates, 9. 64%) and Escherichia coli (5 isolates, 6. 02%). While S. aureus was the most frequent microorganismisolated from wounds, it was isolated from 35 wounded patients(28. 23%) followed by P. aeruginosa 30 isolates (24. 19%), E. coli20 isolates (16. 13%), K. pneumonea 15 isolates (12. 10%), P. vulgaris 12 isolates (9. 68%), Staphylococcus epidermidis 7isolates (5. 65) and Streptococcus pyogenes 4 isolates (3. 23%) (Table 3). The prevalence of bacteria from three hospital universities(Ain-Shams, El-Hussein and Mansoura) is illustrated in Figs. 1–3, respectively. 3. 1. Antibacterial susceptibility patternK. pneumonia isolates were resistant to

amoxicillin and amoxicillin/clavulonic acid in a ratio of 90% while 27. 2% of the isolateswere resistant to ciprofloxacin, 67% to cefaclor, 76% toampicillin, and 63% to imipenim (Fig. 4). Fig. 5 showed thatisolates of P. vulgaris were resistant to cefaclor (75%), imipenimand vancomycin, each (72. 1%), and ciprofloxacin(25%). The resistance of the S. aureus isolates showed that29% of the isolates were resistant to ciprofloxacin, 64% toamoxicillin, 66. 9% to cefaclor and 47% to imipenim (Fig. 6). Ciprofloxacin was found to be the most powerful antibioticand only 17% of E. coli isolates were resistant. However, E. coliisolates 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 amoxicillinand 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 irradiationThe experiment of UV mutant was conducted after preliminarytests 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 ofmutant was increased as the UV doses increased. E. coli andS. pyogenes currently not tolerated UV irradiation over 90 s. The most tolerant organism to UV was S. epidermidis(3. 7 · 102) followed by S. aureus (2 · 102) and P. vulgaris(1 · 102) CFU/ml. Eight isolates of P. aeruginosa were tested against differentantimicrobial agents; two of them were different antibiotic sensitivitypatterns. Plasmids of these eight strains were analyzedand results

revealed that all the six P. aeruginosa isolatescontained the same plasmid pattern. On the other hand, P. aeruginosa isolates (two isolates) that were sensitive to theabove mentioned antibiotic was used as a control and gavethe same plasmid pattern. Fig. 9 shows photograph of plasmid analysis as follows: Pattern 1: (two isolates) which were resistant to ciprofloxacinand amoxicillin; lanes 1 and 2. Pattern II: (one isolate) showsresistance to amoxycillin; lane 3. Pattern III: (two isolates), shows resistance to amoxicillin and cefaclor; lanes 4 and 5. Pattern 4: (one isolate), which was resistant to impenim, ciprofloxacin, and amoxicillin; lane 6. The other two lanes: 7 and 8represented two sensitive isolates. 4. DiscussionNosocomial infections play a role in quality and control inhealth care. Surveillance of these infections is the only wayto gain more insight into their frequency and cause (Beaujeanet al., 2002). Surgical site infections are a problem in all fieldsof surgery (Steinbrecher et al., 1992). In addition, burned patientsare at a high risk for nosocomial infections by multiresistantbacteria, a large proportion of which are gram negative(Mokaddas et al., 1998). Within 24 h, burned patients can startsuffering from opportunistic bacterial attacks that can varyfrom simple infections, such as those easily treatable by antibioticsto more complicated bacteria, which may have natural oracquired resistance to drugs. Infection by multiple drug resistantbacteria could create additional complexity to the problem(Ahmad, 2002). Hussein et al. (1989) stated that the infection at the burnunit was 84. 9%. The same result obtained by Mago (2009)who made a burn sepsis revealed that bacterial colonizaionreached 80. 6%; also, Cremer et al. (1996) found the infectionin burn unit was 94%. The incidence of bacterial infection ofburned patients obtained from El-Hussein University

Hospitaland Mansoura University Hospital was 73. 33% and 52. 94%, respectively. Our results obtained agreed with other workersincluding Hussein et al. (1989), Cremer et al. (1996) and Mago(2009) who stated that the infection at the burn unit was84. 9%, 94% and 80. 6%, respectively. Naeem et al. (2006) atthe burns centre, totally agreed with the result obtained fromMansoura University Hospital where the incidence of burninfection was 10. 1%. The marked reduction in the percentageof infection in Mansoura University Hospital may be attributedto the advanced surgical techniques, instruments andthe precise application of aseptic technique. P. aeruginosa was the most common organism encounteredin burn infection (n= 30, 36. 14%) as indicated in (Table 3). The obtained results agreed with Cremer et al. (1996) andBranski et al. (2009) who found P. aeruginosa in burn infectionin a percentage of 49% and 53. 9%, respectively. P. aeruginosaremains a significant pathogen in burn infection, its pathogenicitybeing associated with the production of a cocktail ofvirulence determinants, which is regulated by a population–density-dependant mechanism and diffusion of signalingmolecules in the burn-wound environment (Koeber et al., 2002). The high predominance of P. aeruginosa among theburned patients must reflect a proper attention to the woundsof the burned patients. On other hand, the isolated P. aeruginosa were at a lowerfrequency rate as in Ain-Shams Hospital where P. aeruginosaIncidence of 20% of burn exudates (Saleh, 2000). Also, Kluyatmans (2007) isolated P. aeruginosa from burn exudatesat frequencies of 19. 7% and 21%, respectively. The second most important microorganism isolated fromburned patients was S. aureus (n = 25, 30. 12%). Husseinet al.

(1989) and Mohamed et al. (2000) also isolated S. aureusas the second most important microorganism encountered inburned patients following P. aeruginosa, S. aureus andS. pyogenes that were found to tolerate sunlight up to120 min of exposure. These results agreed with Mohamed et al. (2000) who isolatedK. pneumonia and E. coli from burn infections at frequenciesof 2% and 7%, respectively in addition to Prot. mirabilis. On the other hand, this result was not in agreement withKhashaba (1981) who found that the most predominant isolatedorganism was S. aureus (53. 8%), followed by Klebsiellasp. (26. 3%), P. aeruginosa (18. 8%), Proteus sp. (6. 3) andE. coli (3. 8%). On the other hand, this result was not in agreementwith Khashaba (1981) who found that the most predominantisolated organism was S. aureus (53. 8%), followed byKlebsiella 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 variationsin both environmental conditions and attitudes towardmanagement of the burn wound. The number of wound infectionsinvolved was 365 patients (150 patients from Ain-ShamsUniversity Hospital, 30 patients from Mansoura UniversityHospital, and 185 patients from El-Hussein University Hospital). Out of 30 patients from Mansoura University Hospital, 25cases (83. 33%) developed bacterial infection and out of 185patients from surgery department at El-Hussein Hospital, 125 patients (67. 57%) developed bacterial infections. This resultwas in accordance with Aganovic et al. (1994) whose rateof nosocomial infection of post-operative wounds was 69. 45%. On the other hand, post-operative infection was at a lowerrate. Mostafa (2006) found that surgical wound infectionwas the commonest nosocomial infection (40%). Mohamedet al. (2000) found that

the infection rate in postoperativewounds was 37. 3% Our results disagreed with the resultsobtained by Holzheimer et al. (1990), Marroni et al. (2003), Lee et al. (1990) and El-Daghestany (1992). They found thatthe overall nosocomial infection of post-operative was 13%, 2. 1%, 11. 4%, and 11. 1%, respectively, while Nageb (1990), Eltahawy et al. (1992) and Abussaud (1996) found that theoverall incidence of surgical infections was 8. 7%, 9%, and8%, respectively. The most common microorganisms isolated from woundedpatients were S. aureus (n= 35, 28. 23%). The obtained resultmore or less agreed with the result of Mostafa (2006) whofound S. aureus in significantly high prevalence in woundinfection (36. 2%). Also Mohamed et al. (2000) reported thatS. aureus was the most common pathogen responsible forthe post operative wound infection (33. 7%). Different results were obtained by Nageb (1990) and Zaghloul(1993), they isolated S. aureus at a prevalence of 19. 2%and 13. 2% respectively. Other isolated S. aureus at higher frequencyrates: Khozam (1987) (59%) and Master et al. (2010) (88%). The second most important microorganism isolated fromthe wounds was P. aeruginosa (n = 30, 24. 19%). Mohamedet al. (2000) reported that P. aeruginosa was one of the mostcommon pathogens responsible for the post-operative woundinfection (25. 3%), Saleh (2000) and Cestari et al. (1999) isolatedP. aeruginosa from 20% and 31. 2% of surgical woundexudates samples, respectively. Low frequency rate was presentedby Nageb (1990) and Kluyatmans (2007) they isolatedP. aeruginosa from 11. 5% and 8% of surgical wound exudates, respectively. Mohamed et al. (2000) isolated K. pneumonia (18%) fromthe post-operative wound infection. Khozam (1987) foundKlebsiella sp. (9. 9%). Abussaud (1996) isolated Klebsiella sp. at (10%).

The policy of antibiotic treatment was always based onin vitro susceptibility test. P. aeruginosa was always amongthe bacteria most readily acquiring resistance toward antimicrobialdrugs. It could cause septicemia in burned patients. So careful attention should be paid to inpatients whosewounds are colonized by this organism (Ashour, 2000). Seven antimicrobial agents were used which are Amoxycillin, Cefaclor, Imipenim, Amoxycillin/clavulonic acid, Ciprofloxacin, Vancomycin, and Ampicillin. Amoxycillin, Cefaclor, were of low activity, and this may beattributed to the extensive use of these drugs. The resistancepattern 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 Cefaclorwas as follows: P. aeruginosa (100%), E. coli (79. 9%), K. pneumonia 67%), Prot. mirabilis (69%), and S. aureus(61%). The resistance pattern to Cefaclor was as follows: P. aeruginosa (100%), E. coli (48%), K. pneumonia (90%), P. vulgaris (75%) and S. aureus (44%). Imipenim was the mostpotent antimicrobial agents against the isolated gram-negativebacilli where 89% of P. aeruginosa, 82% of E. coli, 100% of K. pneumonia and 97. 5% of P. vulgaris isolates were resistant toimipenim. Ciprofloxacin comes after imipenim in activityagainst the isolated Gram-negative bacilli. Imipenim was the most active antibiotic againstP. aeruginosa, El-Naggar (1984), Mohamed et al. (2000) andSaleh (2000), they demonstrated the resistance of P. aeruginosaisolates to imipenim were 98%, 85% and 100%, respectively. Survey at the North of Portugal was performed to assess thelevel of susceptibility to the most common antibiotics with anti-pseudomonal activity against P. aeruginosa. It revealedthat out of 525, 10% of the isolates were resistant to

imipenim(Gardoso et al., 2002). Saleh (2000) found that 64% ofP. aeruginosa strains were resistant to ciprofloxacin El-Daker(2002) found that imipenim and ciprofloxacin were the mosteffective antibiotics toward multidrug resistant Gram-negativebacilli at Ain-Shams University Hospitals. Hussein et al.(2001) investigated that the sensitivity rate for imipenim was71% and for ciprofloxacin 52%, in contrast most of the testedisolates showed resistance to the third generation Amoxycillin/clavulonic acid (90%). The prevalence of Cefaclor-resistant P. aeruginosa inThailand was 24%, which is higher than the value reportedfor P. aeruginosa isolated of North America and Europe(Girlich et al., 2002). Vancomycin was the effective drug insurgical and medical patients infected with methicillin-resistantS. aureus isolated from Ain-Shams University Hospitals. Susceptibilitytesting of isolates S. aureus collected from differenthospitals in Riyadh, Saudi Arabia to vancomycin showed thatall isolates were sensitive (Fouda et al., 2005). The effect of UV radiation on isolated bacteria was carriedout to figure out the role of UV to control the growth of thebacteria contaminating the burns and wounds. The lethal effectof UV on the isolated bacteria varied considerably amongthe species examined, e. g., it is as low as 90 s in the case ofE. coli and S. pyogenes, whereas it is as high as 150 s in caseof S. aureus, P. vulgaris and S. epidermidis. Bacteria that wereresistant to UV rays and endured their effect for up to 150 smay be characterized by a DNA with a high GC mol.% valueand consequently, low thymine content, thus reducing theprobability for thymine dimerization. Bacteria may also exhibithigh activity and complete repair systems for photo reactivation, excision repair and post-replication repair. In the present work six multi-drug resistant and

two sensitivestrains of P. aeruginosa were selected for study concerningtheir plasmid profile. They were tested against seven antimicrobialagents. The P. aeruginosa isolates revealed five differentantibiotic patterns. The result revealed that all the eight P. aeruginosa isolates contained the same plasmid pattern, whichmeans that there was no correlation between plasmid patternand their antimicrobial activity. This result was in agreementwith that of Fouda et al. (2005).