Bacterial Isolates and Their Antibiograms of Burn Wound Infections in Burns Specialist Hospital in Baghdad

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Abstract:

A total of 54 out of 67 (80.59%) of burn wound swab showed growth of one, or two, or three bacterial pathogens. *Pseudomonas aeruginosa* was the commonest pathogen, isolated in 48.14% of swab samples, followed by *Klebsiella pneumoniae* (31.48%), *Staphylococcus aureus* (27.77%), *Acinetobacter baumanii* (14.81%), *Escherichia coli* (7.40%), and *Citrobacter freundii, Providencia stuartii, Enterobacter cloacae*, with 1.85% isolation percentage for each. All bacterial isolates were tested against 19 antibiotics, and showed multi-drug resistance to 10 antibiotics, or more. The most effective antibiotics were the fifth-generation cephalosporin, ceftobiprole, and and antibiotic combinations, as Ceftazidime / clavulanic acid, and Cefoperazone /sulbactam, and newer generation fluoroquinolone, levofloxacin, and gemifloxacin, which are attractive candidates to be the basic antibiotics in establishment of new hospital policy in Iraq for treatment of burn wound infection of multi-drug resistant bacteria.

Key words: Burn wound infections, Drug-resistance, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylcoccus aureus*, *Acinetobacter baumanii*

Introduction:

Burn wound infection is a problematic one because it delays healing, encourages scarring and may result in bacteremia, sepsis or multiple-organ dysfunction syndrome (a.k.a. organ failure) whereby organs from several systems are unable to maintain homeostasis on their own, requiring immediate medical attention [1]. Bacteria and fungi are the most common pathogens of burn wounds. These microbes form multi-species biofilms on burn wounds within 48 – 72 hours of injury [1]. Organisms originate from the patient's own skin, gut and respiratory flora, as well as from contact with contaminated health care environments and workers [1, 2]. Gram-positive bacteria are some of the first to colonize burns, followed quickly by gram-negative. Fungal infection tends to occur in the later stages after the majority of bacteria have been eliminated by topical antibiotics [1].This study was aimed to isolate bacterial isolates from burn wound infections, and test their antibiotic susceptibility pattern against available antibiotics and newer antibiotic combinations in order to formulate antibiotic policy for better management for these infections.

Materials and Methods:

Samples collection. A total of 67 burn wound samples were taken from burn patients (32 males, 35 females; m/f ratio= 0.91/1) of Burn Specialist Hospital in Medical city directory, Baghdad. The patients were aged between 10 moths and 51 yr (mean, 26). The aetiologies of the burn trauma were flame (37/62= 55.2%), terrorist blasts (16/76= 23.8%), hot liquid (water, tea, cocking oil) (12/67=

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16.9%), electricity (1/67= 1.49%), and chemical agent (1/67= 1.49%).

When samples were collected, special attention was paid to areas where infection was most evident, before dressing changes. The oral, genital, scalp, and anal regions were never used for sample collection. The areas most preferred were the upper and lower extremities. All specimens were inoculated on 5% blood agar, McConkey agar (Himedia, India), and incubated overnight at 37ºC aerobically.

Bacterial pathogens were identified by colonial morphology, slide morphology, Gram reaction, conventional biochemical methods according to standard microbiological techniques [3], Api 20E, and Api Staph bacterial identification test strips (bioMérieux, France).Antimicrobial susceptibility was performed on Mueller-Hinton agar (Himedia, India) by the standard disk diffusion method recommended by the National committee for clinical laboratory standards [4]. The antibiotics tested were: Amoxicillin (10 µg), piperacillin (100 µg), carbenicillin (100 µg), erythromycin (15 µg), Azithromycin (15µg), vancomycin (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cefepime (30 µg), (ceftazidime/ clavulanic acid (30/10 µg), cefoperazone /sulbactam (75/30 µg), amikacin (30 mg), ciprofloxacin (5 mg), norfloxacin (5 µg), levofloxacin (5µg), gemifloxacin (5µg), imipenem (10 µg) and meronem (10 µg) were used. The source for media and antibiotic discs was Hi-Media Ltd. India. Ceftobiprole antibiotic discs (30 µg) was kindly provided by Dr. Visanu Thamlikitlul from Division of Infectious Disease and Tropical Medicine, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

Standard strains *Escherichia coli* MM294, and *Staphylococcus aureus* ATCC 25923 were used as controls.

Results:

A total of 67 burn wound samples were taken from burn patients (32 males, 35 females; m/f ratio= 0.91/1) of Burn Specialist Hospital in Medical city directory, Baghdad. The patients were aged between 10 moths and 51 yr (mean, 26). The aetiologies of the burn trauma were flame (37/67= 55.2%), terrorist blasts (16/67= 23.8%), hot liquid (water, tea, cocking oil) (12/67= 16.9%), electricity (1/67= 1.49%), and chemical agent (1/67= 1.49%) (Figure 1).

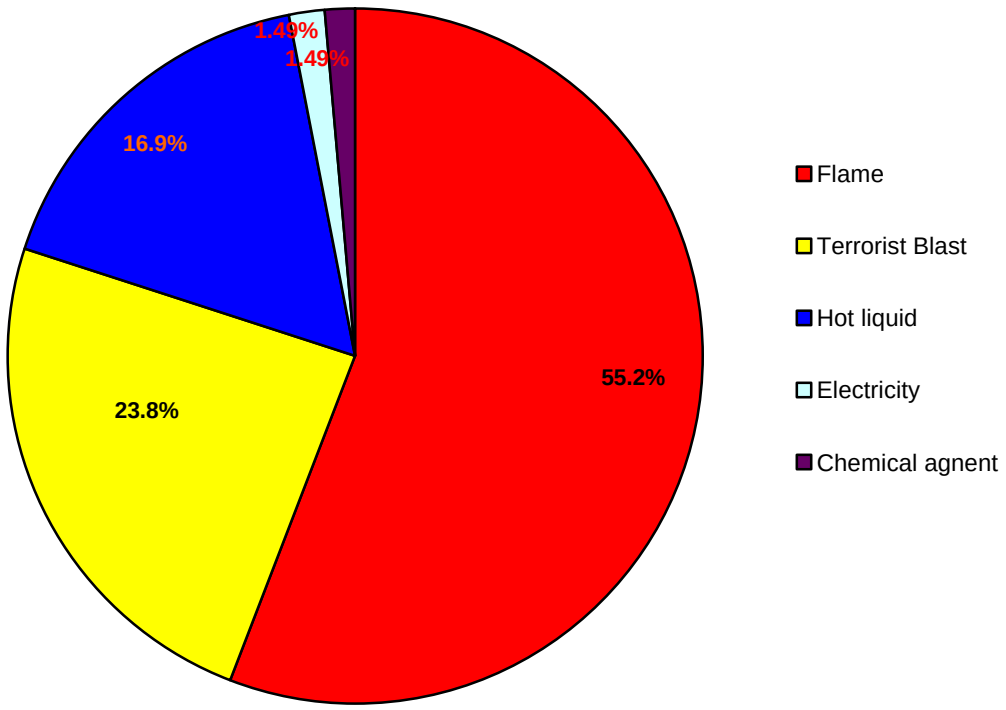

Figure 1. The aetiologies of the burn trauma of 67 burn wound cases admitted in Burn Specialist Hospital in Medical city directory, Baghdad from 11/7/2011 to 15/9/2011.

Culture results.

Bacterial isolates were found in 54 (80.59%) samples and 13 wound swabs were sterile (19.4%). *Pseudomonas aeruginosa* was the commonest pathogen isolated (47.36%), followed by *Klebsiella pneumoniae* (31.48%), *Staphylococcus aureus* (27.77%), *Acinetobacter baumanii*(14.81%), *Escherichia coli* (7.40%), and 1.85% for *Providencia stuartii*, *Citrobacter freundii*, and *Enterobacter* spp, as shown in table 1.

Table 1. Number and percentage of isolates and isolation rate for each organism from burn wound swabs.

No.	Organism	No. of isolates (Rate of isolation)
1	*Pseudomonas aeruginosa*	26/54 (48.14%)
2	*Klebsiella pneumoniae*	17/54 (31.48%)
3	*Staphylococcus aureus*	15/54 (27.77%)
4	*Acinetobacter baumanii*	8/54 (14.81%)
5	*Escherichia coli*	4/54 (7.40%)
6	*Citrobacter freundii*	1/54 (1.85%)
7	*Providencia stuartii*	1/54 (1.85%)
8	*Enterobacter cloacae*	1/54 (1.85%)

Pseudomonas aeruginosa was isolated alone in 12 samples, whereas in concomitant with other pathogens in 14 samples. *K. pneumoniae* was

isolated alone in 7 samples, where in concomitant with other pathogens in 10 samples. *S. aureus* was isolated alone 9 samples, whereas in concomitant with other pathogens in 6 samples. *A. baumanii* was isolated alone in 3 samples, whereas in concomitant with other pathogens in 5 samples. Each of *E. cloacae* and *P. stuartii* was isolated alone in one sample. Other bacterial pathogens were isolated in concomitant with other bacterial pathogens. Figure 2 showed number of samples showed growth of each bacterial species alone to the total number of samples.

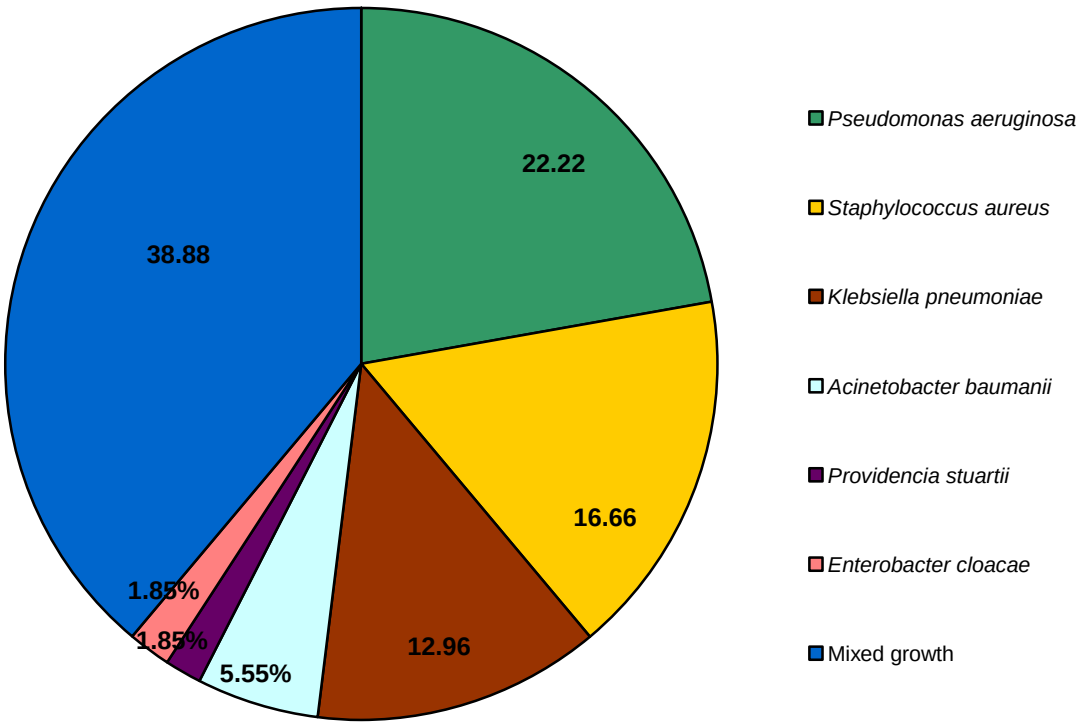

Figure 2. Number of samples showed growth of one bacterial species alone to the total number of samples.

Antibiotic susceptibility tests.

Bacterial pathogens isolated from burn wound infections showed antibiotic susceptibility patterns listed in table 2. All eight bacterial burn wound infections pathogens listed in table 1 were showed multi-drug resistance for 10 antibiotics, or more. *P. aeruginoas* was the most drug-resistant pathogen of bacterial isolates tested. It showed resistance to third generation cephalosporin of 100% and 88.96% for Cefotaxime, and Ceftriaxone, respectively, whereas moderate resistant to combination of this generation of Ceftazidime / clavulanic acid (46.15), and Cefoperazone /sulbactam (53.48). *P. earuginosa* was less resistant to the fourth-generation cephalosporins, Cefipime, with 76.92% resistance, wherea more sensitive to the fith-generation cephalosporin, Ceftobiprole, with only 19.23% resistance.

Pseudomonas. aeruginosa was most sensitive to Imipinem, and Meronem, with resistant of 30.76%, and 23.07%, respectively. *P. aeruginosa* was resistant to old fluoroquinolones as ciprofloxacin, and norfloxacin, as well as newer generation fluoroquinolones as levofloxacin, and gemifloxacin.

Klebsiella pneumoniae was resistant to the cephalosporin third and fourth-generation, with resistance of 100%, 88.46%, and 76.92 for cefotaxime, ceftriaxone, and cefepime, respectively, whereas sensitive to cephalosporin fifth-generation, ceftobiprole, with resistance of only 19.23%. *K. pneumoniae* showed sensitivity to cephalosporin third-generation combination, as Ceftazidime / clavulanic acid, and Cefoperazone /sulbactam, with 29.41%, and 17.64% resistance, respectively. *K. pneumoniae* was high sensitive to imipinem, and meronem, with only resistance of 6.66% for each, and resistant to ciprofloxacin (70.58%), and norfloxacin (70.58%), but more sensitive to newer fluoroquinolone generation, levofloxacin (41.17%), and gemifloxacin (11.76%).

Staphylococcus. aureus was highly resistant to penicillins, as amoxicillin (100%), piperacillin (93.33%), and carbenicillin (86.66%). It was resistant to third-generation cephalosporin, cefotaxime, and ceftriaxone, with resistance of 93.33% for each, whereas low resistant to their combinations with other antibiotics, Ceftazidime / clavulanic acid and Cefoperazone /sulbactam, with only 14.28% resistance. *S. aureus* showed moderate resistance to fourth-generation cephalosporin, cefepime (53.33%), whereas very low resistant to fifth-generation cephalosporin, ceftobiprole (6.66%). *S. aureus* showed moderate resistance for old flouroquinolone, ciprofloxacin (53.33%), and norfloxacin (46.66%), whereas less resistant to new generation flouroquinolone, levofloxacin (33.33%), and gemifloxacin (33.33%). *S. aureus* showed to be totally resistant to vancomycin.*Acinetobacter. baumanii* was resistant to the cephalosporin third and fourth-generation, with resistance of 87.5 %, 87.5 %, and 62.5% for cefotaxime, ceftriaxone, and cefepime, respectively, whereas moderately resistant to cephalosporin fifth-generation, ceftobiprole, with resistance of only 25%. Also, *A. baumanii* showed low resistant to cephalosporin third-generation antibiotic combination with other antibiotics, as Ceftazidime / clavulanic acid and Cefoperazone /sulbactam, with only 37.5 % resistance. *A. baumanii* showed no resistance to imipinem, meronem compared with *P. aeruginosa*, *K. pneumoniae*, or *S. aureus* and their resistance to fluoroquinolone was less than *P. aeruginosa*, *K. pneumoniae*, and had mild resistance to new generation flouroquinolone, levofloxacin (12.5%), and gemifloxacin (12.5%).Other bacterial pathogens showed different antibiotic susceptibility pattern, as *P. stuartii* found to be resistant (100%) to old, and new generation of fluoroquinolone, whereas Enterobacter cloacae found to be non- resistant (0%) to fluoroquinolone.*P. stuartii*, and *E. cloacae* were found to be resistant (100%) to third, fourth-generation cephalosporin, whereas non-resistant to fith-generation cephalosporin. Also, *P. stuartii*, and *E. cloacae* were found to be non-resistant to cephalosporin third-generation antibiotic combination with other antibiotics, as Ceftazidime / clavulanic acid and Cefoperazone /sulbactam.

Escherichia. coli was also multi-drug resistant, but less resistant pathogen to

antibiotics tested in comparing with other pathogens.

Table 2. Antibiograms for Gram-positive and Gram-negative bacterial isolates isolated from burn wound infection by disc diffusion methed.

No.	**Antibiotic (disc content)**	Percent resistance (No. of resistant bacterial isolates/ No. of bacteria tested)							
		Pseudomonas aeruginosa	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Acinetobacter* spp.	*Escherichia coli*	*Citrobacter freundii*	*Providencia stuartii*	*Enterobacter cloacae*
1	Amoxycillin (10 mg)	N.D.	100 (17/17)	100 (15/15)	100 (8/8)	100 (4/4)	100 (1/1)	100 (1/1)	100 (1/1)
2	Piperacillin (100 mg)	N.D.	N.D.	93.33 (14/15)	N.D.	N.D.	N.D.	N.D.	N.D.
3	Carbenicillin (100 mg)	N.D.	N.D.	86.66 (13/15)	N.D.	N.D.	N.D.	N.D.	N.D.
4	ciprofloxacin (5 mg)	84.6 (22/26)	70.58 (12/17)	53.33 (8/15)	62.5 (5/8)	50 (2/4)	100 (1/1)	100 (1/1)	0 (0/1)
5	Norfloxacin (10 µg)	76.92 (20/26)	70.58 (12/17)	46.66 (7/15)	62.5 (5/8)	50 (2/4)	100 (1/1)	100 (1/1)	0 (0/1)
6	Levofloxacin (5µg)	69.23 (18/26)	41.17 (7/17)	33.33 (5/15)	12.5 (1/8)	0 (0/4)	0 (0/1)	100 (1/1)	0 (0/1)
7	Gemifloxacin (5µg)	65.38 (17/26)	11.76 (2/17)	33.33 (5/15)	12.5 (1/8)	0 (0/4)	0 (0/1)	100 (1/1)	0 (0/1)
8	Erythromycin (15 µg)	100 (26/26)	88.23 (15/17)	100 (15/15)	100 (8/8)	100 (4/4)	100 (1/1)	100 (1/1)	100 (1/1)
9	Azithromycin (15µg)	92.3 (24/26)	70.58 (12/17)	93.33 (14/15)	87.8 (7/8)	100 (4/4)	100 (1/1)	100 (1/1)	100 (1/1)
10	Vancomycin (30 µg)	N.D.	N.D.	100 (15/15	N.D.	N.D.	N.D.	N.D.	N.D.

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11	Cefotaxime (30 µg)	100 (26/26)	100 (17/17)	93.33 (14/15)	87.5 (7/8)	100 (4/4)	100 (1/1)	100 (1/1)	100 (1/1)
12	Ceftriaxone (30 µg)	88.46 (23/26)	82.35 (14/17)	93.33 (14/15)	87.5 (7/8)	100 (4/4)	100 (1/1)	100 (1/1)	100 (1/1)
13	Cefepime (30 µg)	76.92 (20/26)	58.82 (10/17)	53.33 (8/15)	62.5 (5/8)	50 (2/2)	0 (0/1)	100 (1/1)	100 (1/1)
14	Ceftobiprole	19.23 (5/26)	23.52 (4/17)	6.66 (1/15)	25 (2/8)	0 (0/4)	0 (0/1)	0 (0/1)	0 (0/1)
15	Ceftazidime / clavulanic acid (30/10 µg)	46.15 (12/26)	29.41 (5/17)	14.28 (2/15)	37.5 (3/8)	25 (1/4)	0 (0/1)	100 (1/1)	0 (0/1)
16	Cefoperazone /sulbactam (75/30 µg)	53.48 (14/26)	17.64 (3/17)	14.28 (2/15)	37.5 (3/8)	25 (1/4)	0 (0/1)	100 (1/1)	0 (0/1)
17	Imipenem (10 µg)	30.76 (8/26)	11.76 (2/17)	6.66 (1/15)	0 (0/8)	0 (0/4)	0 (0/1)	0 (0/1)	0 (0/1)
18	Meronem (10 µg)	23.07 (6/26)	5.88 (1/17)	6.66 (1/15)	0 (0/8)	0 (0/4)	0 (0/1)	0 (0/1)	0 (0/1)
19	amikacin (30 µg)	84.61 (22/26)	76.47 (13/17)	73.33 (11/15)	75 (6/8)	75 (3/4)	100 (1/1)	100 (1/1)	0 (0/1)

N.D.: Not Done.

Discussion:

P. aeruginosa was the most common pathogen of burn wound infection of this study, as isolated in 48.14% (26/54) of burn wound infection cases.

The prevalence of *P. aeruginosa* in such cases is resulted from surviving well in hospital environment. Once it was established, it can persist for months within a unit, posing as multi-drug resistant nosocomial infection risk for patients being treated there [5; 6].

The prevalence of *P. aeruginosa* in burn wound infection cases was documented in several studies worldwide, as Arslan *et al*. [7], Mehta *et al*. [8], and Estahbanati *et al*. [9].

K. pneumoniae was the second frequent pathogen of burn wound infection, and isolated from 31.48% (17/54) of burn wound infections. *K. pneumoniae* is one of the most important nosocomially acquired pathogens [10], and one of the most frequent burn wound infection pathogens, as Kehinde *et al*. [11] showed that *K. pneumoniae* was the most frequent pathogen (34.4%) of burn wound infection, followed by *P. aeruginosa* (29.0%) and *S. aureus* (26.8%).

S. aureus was the third frequent pathogen of burn wound infection, and isolated from 27.77% (15/54) of burn wound infections. *S. aureus* is one of the most common causes of nosocomial infections, and responsible for most nosocomial infections, including burn wound infections [12].

The most striking result of antibiotic susceptibility tests is that all bacterial isolates were multi-drug resistant, which showed resistance to 10

antibiotics, or more, that is the cause of high mortality rate of burn wound infections, and their complications, as bacteraemia, and septicaemia [13].

All bacterial pathogens isolated from burn wound infections were showed to have high-level resistance to third-generation cephalosporin, and moderate to high-level resistance to fourth-generation cephalosporin.

For many years, the third and fourth-generation cephalosporins have been utilitzed in the treatment of a broad range of infections. The reduction in efficacy of these antimicrobials in hospitals seen in recent years as a result of the development of resistance to these compounds [14].

Ceftobiprole showed high activity against burn wound infections pathogens, that have been showed low resistance against this antibiotic (Table 2), compared with high resistance to the third, and fourth-generation cephalosporins, cefotaxime, and ceftriaxone (Table2).

In clinical trials, ceftobiprole demonstrated high cure rates in patients with complicated skin infections, including the potentially deadly "super bug," methicillin-resistant *S. aureus* (MRSA), and showed broad-spectrum activity against Gram-positive and Gram-negative bacteria. Ceftobiprole was well tolerated with common treatment-emergent adverse events, including nausea, taste disturbance, diarrhea and vomiting [15].

Fifth-generation cephalosporins are attractive candidates to replace third and fourth-generation cephalosporins for the treatment of many serious infections, including burn wound infections.

Combination of third-generation cephalosporins with other antibiotics, Ceftazidime / clavulanic acid and Cefoperazone /sulbactam showed high activity against Gram-negative and Gram-positive isolates, and they are excellent candidates to replace the third and fourth-generation cephalosporins, that are used widely in clinical practice in our hospitals, including burn wound infections, but all isolates showed high resistance against. High activity of Ceftazidime / clavulanic acid, and cefoperazone /sulbactam belongs to their high stability to β-lactamases [16; 17].

Imipenem and meronem are ß- lactam antibiotics that have broad-spectrum activity against Gram-negative and Gram-positive bacteria [18]. All bacterial isolates showed low resistance to these antibiotics, and most isolates of Enterobacteriaceae showed no resistance to these antibiotics.

This could be due to reason that these are reserve drugs and used as last options for multi-drug resistant bacteria in our hospital settings.

All bacterial isolates showed moderate to high resistance to old fluoroquinolone, ciprofloxacin, and norfloxacin, but moderate to no resistance to newer generation fluoroquinolone, levofloxacin, and gemifloxacin.

Newer generation flouroquinolone have proven themselves to be effective agents across the full gamut of skin and skin structure infections, clinicians should be prudent in the use of flouroquinolones as first line agents. Their efficacy against a broad variety of less common Gram-negatives for which current antimicrobial choices are limited and dwindling needs to be preserved. The utility of these agents lies in their ability to serve as monotherapy in the face of polymicrobial infections, as burn wound infection, where Gram-negative organisms are suspected along with the usual gram positive culprits. It provides an additional benefit in its coverage of anaerobes, and its role in

the treatment of these infections is likely to expand [19].

It is recommended to establish new policy for antibiotic treatment in cases on burn wound infection, based on regular screening on antibiograms of burn wound infection pathogens.

Fifth-generation cephalosporin, ceftobiprole, and antibiotic combinations, as Ceftazidime / clavulanic acid, and Cefoperazone /sulbactam, and newer generation fluoroquinolone, levofloxacin, and gemifloxacin, are attractive candidates to occupy priority in new hospital policy for treatment of burn wound infection of multi-drug resistant bacteria.

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المُمْرِضات البكتيرية لأخماج جروح الحروق وأنماط حساسيتها الدوائية في مستشفى الحروق التخصصي في بغداد

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الخلاصة:

أظهرت نتائج الزرع لـ67 مسحة خمج جروح الحروق أن 54 منها (80.59%) أعطت نموّاً بكتيرياً لواحد، أو إثنين، أو ثلاث ممرضات بكتيرية. كانت *Pseudomonas aeruginosa* هي الأكثر شيوعاً فيها، إذ عزلت في 48.14% من المسحات، ومن بعدها *Klebsiella pneumoniae* (31.48%)، *Staphylococcus aureus* (27.77%)، *Acinetobacter baumanii* (14.81%)، *Escherichia coli* (7.40%)، ثم *Citrobacter freundii*، *Providencia stuartii*، و *Enterobacter cloacae* بنسبة عزل 1.85% لكل منها. أختبرت العزلات البكتيرية تجاه 19 مضاد حيوية، وكانت جميعها مقاومة لـ10 مضادات حيوية، أو أكثر. أكثر مضادات الحيوية فعالية كانت الجيل الخامس للسيفالوسبورينات، ceftobiprole، وخلائط مضادات الحيوية، Ceftazidime/clavulanic acid، و Cefoperazone/sulbactam، وجيل الفلوروكوينولات الأحدث، levofloxacin، و gemifloxacin، والتي تعد مرشّحات واعدة لتكون الأساس في وضع سياسة جديدة للمستشفيات في العراق لعلاج أخماج جروح الحروق الناتجة عن البكتريا متعددة المقاومة لمضادات الحيوية.