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Original article

Efficiency of chromogenic medium (HiChrome universal medium) for identification of organisms causing burn wound infection and their pattern of antimicrobial susceptibility at Ain Shams University Hospitals

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ABSTRACT

Background: Burn wound infections are one of the most dangerous burn complications because they are a leading cause of morbidity and mortality in burn patients. Their successful treatment necessitates the rapid isolation and identification of causative species using antibiotic susceptibility patterns that are acceptable. **Aims and objectives:** To evaluate the efficiency of the chromogenic medium (**HiChrome universal medium**) in comparison to conventional method in identification of organisms causing burn wound infections regarding time, accuracy and cost and profiling of antimicrobial susceptibility patterns of burn wound isolates. **Methods:** Eighty-three wound samples were collected from inpatient and ICU patients at Ain Sham University Burn Unit. All the wound swabs were analysed using conventional media and chromogenic medium (HiCrome Universal medium) to compare between their results. Antibiotic sensitivity testing was done using disk diffusion method. **Results:** HiChrome universal medium was effective and could identify all organisms in the obtained samples within 24 hours and its efficiency was almost the same as conventional method including (nutrient, blood and MacConkey' s agar medium followed by biochemical tests) but more rapid. Out of the 83 swabs taken, positive growth was detected in 81 swabs (97.5%). Out of this, Gram negative organisms were isolated from 45(54.2%) isolates while 21(25.3%) grew solely Gram positive organisms. However 15(18.1%) grew mixed Gram positive and negative organisms. *Staphylococcus aureus* (30.1%) was the commonest among Gram positive organisms 25(30.1%) and *Pseudomonas aeruginosa* 38(45.8%) was the commonest among Gram negative organisms. Vancomycin was the most effective antibiotic against Gram positive bacteria and Imipenem was the most effective against Gram negative organisms. **Conclusion:** HiCrome Universal agar can be used to quickly isolate all organisms with low cost.

Introduction

Burn wound infection continues to be a major issue of concern in developing countries [1]. Infections in burn patients are caused by a number of factors, including an exposed body surface, an immunocompromised state, invasive procedures

conducted in the health care facility and a long stay in the hospital [2]. Organisms usually infect burn wounds causing delay in wound healing [3]. The wound is initially colonized by Gram-positive bacteria such as *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*

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followed by Gram negative bacterial infection such as *Pseudomonas aeruginosa*, *E. coli* and *klebsiella pneumoniae* [4].

Identification of organisms has used to be done by conventional methods especially in the developing countries [5]. However, none of the conventional media including blood agar, nutrient agar and MacConkey agar media can support the growth and identification of different wound isolates without performing different biochemical tests which is time and money consuming [6].

The chromogenic media can reduce the number of these confirmatory tests and they are well adapted for identification of samples containing mixed organisms [7]. HiChrome universal differential medium is a type of chromogenic media that will help in isolation of most of organisms causing wound infection [8]. The pathogens grow as coloured colonies due to their enzymatic effects on substrates present in the chromogenic agar medium [6].

Methodology

This Observational cross sectional study was performed in the Medical Microbiology and Immunology Department, Faculty of Medicine, Ain Shams University and was approved by the Research Ethics Committee with approval number (FWA 000017585) at the period between November 2020 to March 2021.

Patient selection and collection of samples

This study was conducted on eighty-three patients admitted to the Burn Unit (inpatients and ICU) at Ain Shams University Burn Unit. Informed consent were obtained from the patients or from their

relatives after explaining the study and its goals to them. All patients were analysed regarding age, sex, C-reactive proteins(CRP) and lymphocytic count.

Inclusion criteria

Burn patients (inpatients and ICU) with burn wound infection at Burn Unit of Ain Shams University after five days of hospitalization.

Exclusion criteria

- Patients without burn wound infection.
- Patients with burn wound period less than 5 days.

Study procedures

All burn wound swabs were subjected to the following tests:

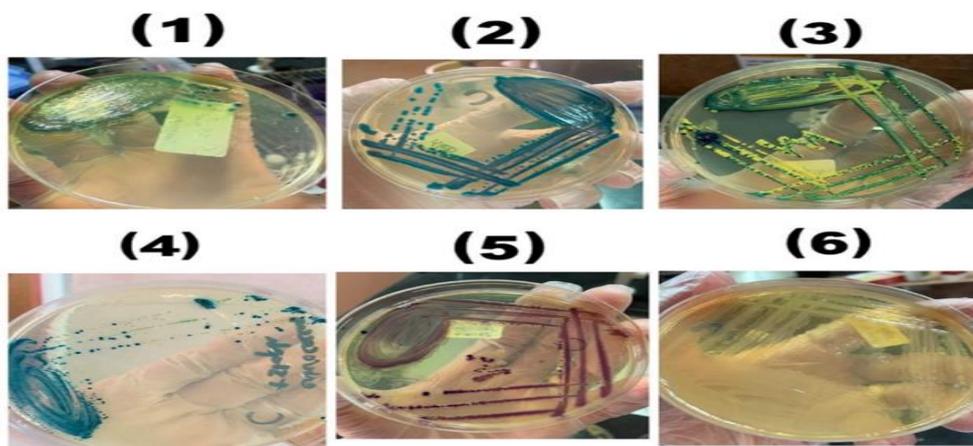
• Conventional method: Wound swab samples were inoculated on nutrient ,blood and MacConkey agar media, incubated at 35-37 celsius under aerobic conditions according to **Collee et al.** [9].

• Colorimetric method: Wound swab samples were inoculated on the Hichrome universal media (Himedia-India), incubated at 35-37 celsius under aerobic conditions and colonies were identified according to colour changes by **Perry & John** [10]. *Pseudomonas aeruginosa* showed greenish colonies, *Klebsiella pneumoniae* showed large blue mucoid colonies, *Staphylococcus aureus* showed yellow colonies, *Micrococcus leuteus* showed blue colonies, *Enterococcus faecalis* showed blue colonies, *E coli* showed purple colonies and *Proteus vulgaris* showed light brown colonies.

Antimicrobial susceptibility patterns of the isolated strains using disc diffusion were performed according to clinical and laboratory standards institute (CLSI) [11].

Identification of the wound isolates

Figure 1. shows different colony colours on the Hichrome universal media as in the following:



- (1) Greenish colonies of *Pseudomonas aeruginosa*. (2) Large blue mucoid colonies of *Klebsiella pneumoniae*. (3) Yellow colonies of *Staphylococcus aureus* mixed with blue colonies of *Micrococcus leuteus*. (4) Small blue colonies of *Enterococcus faecalis*. (5) Purple colonies of *E. coli*. (6) Light brown colonies of *Proteus vulgaris*.

Results

This study was conducted on 83 burn cases admitted to Ain Shams University hospital in the period from November 2020 to March 2021. They were 45 females (54.2%) and 38 males (45.8%) with age ranged from 10 months to 70 years with median age of 21.

Regarding the cause of burn, scalds was the cause of injury for 48 patients (57.8%), flame was responsible for 21 injuries (25.3%) and 11 patients each (13.3%) were injured by chemicals and 3 patients (3.6%) were injured by electricity. According to the body surface area, it ranged between 9% and 40%, with mean \pm SD 21.16 \pm 7.31%. The mean \pm SD duration of hospitalization was 2.09 \pm 0.80 weeks after injury. Regarding the laboratory tests, C-reactive proteins (CRP) ranged from 4 – 61mg/dl with median value of 15mg/dl and lymphocytic count ranged from 19 – 64 lymphocytes/mcl with mean \pm SD 37.70 \pm 10.31% as shown in **table (1)**.

Regarding the isolated species

Organisms isolated by chromogenic media after 24 hours among the studied cases

The predominant bacteria was *Pseudomonas aeruginosa* (45.8%). It was followed by *Staphylococcus aureus* (30.1%), *Klebsiella pneumoniae* (15.7%), *Proteus vulgaris* (7.2%), *Enterococcus faecalis* (7.2%), *E coli* (4.8%) *Acinetobacter baumannii* (3.6%) and *Micrococcus* (1.2%). Polymicrobial infection was seen in 15 (18.1%).

Organisms isolated by conventional method after 48 hours among the studied cases

The predominant bacteria was *Pseudomonas aeruginosa* (46%). It was followed by *Staphylococcus aureus* (27.7%), *Klebsiella pneumoniae* (16.9%), *Proteus vulgaris* (7.2%), *Enterococcus faecalis* (4.8%), *E coli* (3.6%) *Acinetobacter baumannii* (3.6%) and *micrococcus leuteus* (1.2%). Polymicrobial infection was seen in 10 (12.0%) samples.

Comparison was done between both methods and it showed that both media had the ability to identify almost the same organisms, however, the chromogenic media was found to identify the isolates within 24 hours compared to 48 hours when conventional media was used as shown in **table (2)**.

Regarding the antibiotic sensitivity

Resistance to various antimicrobial agents was proved to be widespread using disc-diffusion susceptibility method. *Pseudomonas aeruginosa* isolates were resistant to ampicillin (90.0%), ceftriaxone (80.0%), piperacillin/tazobactam (63.3%) and cefepime (83.3%). Most of *Staphylococcus aureus* isolates were sensitive to vancomycin (58.8%) and linezolid (62.5%) and imipenem (100%), and resistant to ampicillin (65%), tazobactam (80.0%), ceftriaxone (80.0%), cefepime (90.0%) gentamycin (65%), ciprofloxacin (78.7%), amikacin (78.7%). The *Klebsiella pneumoniae* and other *Enterobacteriaceae species* isolates were sensitive to imipenem (66.7%), ceftazidime (42.9%), meropenem (42.9%) and resistant to, ampicillin (100%), ceftriaxone (71%), tazobactam (100%), amikacin (85.7%), gentamycin and ciprofloxacin (66.7%) (**Table 3**).

Table 1. Demographic data and characteristics of the studied cases.

		Total no. = 83
Age (yrs)	Median (IQR)	21
	Range	10 months – 70 years
Sex	Females	45 (54.2%)
	Males	38 (45.8%)
Cause of burn	Scalds	48 (57.8%)
	Chemical	11 (13.3%)
	Flame	21 (25.3%)
	Electric	3 (3.6%)
Degree of burn	2nd degree	54 (65.1%)
	3rd degree	29 (34.9%)
Period of burn wound (weeks)	Mean \pm SD	2.09 \pm 0.80
	Range	0.57 – 3.57
Burn surface area	Mean \pm SD	21.16 \pm 7.31
	Range	9 – 40
C-reactive protein(CRP)	Median (IQR)	15
	Range	4 – 61mg/dl
Lymphocytic count	Mean \pm SD	37.70 \pm 10.31
	Range	19 – 64

Table 2. shows comparison between chromogenic media after 24 hours and conventional media after 48 hours in detection of various burn wound organisms. No statistically significant value was detected.

	Organisms isolated by chromogenic media after 24 hours	Organism isolated by conventional method after 48 hours	Test value	p-value	Sig.	Kappa agreement (95% CI)
	No. (%)	No. (%)				
<i>Pseudomonas</i>	38 (45.8%)	37 (44.6%)	0.024*	0.877	NS	0.976 (0.928 to 1.000)
<i>Klebsiella</i>	13 (15.7%)	14 (16.9%)	0.044*	0.834	NS	0.867 (0.721 to 1.000)
<i>Staphylococcus</i>	25 (30.1%)	23 (27.7%)	0.117*	0.732	NS	0.941 (0.861 to 1.000)
<i>Proteus</i>	6 (7.2%)	6 (7.2%)	0.000*	1.000	NS	1.000 (1.000 to 1.000)
<i>Enterococcus</i>	6 (7.2%)	4 (4.8%)	0.426*	0.514	NS	0.788 (0.504 to 1.000)
<i>Ecoli</i>	4 (4.8%)	3 (3.6%)	0.149*	0.699	NS	0.851 (0.564 to 1.000)
<i>Acinetobacter</i>	3 (3.6%)	3 (3.6%)	0.000*	1.000	NS	1.000 (1.000 to 1.000)
<i>Micrococcus</i>	1 (1.2%)	1 (1.2%)	0.000*	1.000	NS	1.000 (1.000 to 1.000)

p-value > 0.05: Non significant; p-value < 0.05: Significant; p-value < 0.01: Highly significant*: Chi-square test

Table 3. shows sensitivity of different antibiotics against the identified burn wound organisms..

		Chromogenic media groups						Test value	p-value	Sig.
		<i>Pseudomonas</i>	<i>Klebsiella</i>	<i>Staphylococcus aureus</i>	<i>Proteus</i>	Others	Combined			
		No. = 30	No. = 7	No. = 20	No. = 4	No. = 5	No. = 15			
AM	Resistant	27 (90.0%)	7 (100.0%)	13 (65.0%)	2 (50.0%)	5 (100.0%)	11 (73.3%)	10.448	0.063	NS
	Sensitive	3 (10.0%)	0 (0.0%)	7 (35.0%)	2 (50.0%)	0 (0.0%)	4 (26.7%)			
	Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
VA	Resistant	0 (0.0%)	0 (0.0%)	7 (41.2%)	0 (0.0%)	0 (0.0%)	2 (50.0%)	0.830	0.660	NS
	Sensitive	0 (0.0%)	0 (0.0%)	10 (58.8%)	0 (0.0%)	1 (100.0%)	2 (50.0%)			
	Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
Tpz	Resistant	19 (63.3%)	7 (100.0%)	16 (80.0%)	4 (100.0%)	5 (100.0%)	10 (66.7%)	8.399	0.136	NS
	Sensitive	11 (36.7%)	0 (0.0%)	4 (20.0%)	0 (0.0%)	0 (0.0%)	5 (33.3%)			
	Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
LNZ	Resistant	0 (0.0%)	0 (0.0%)	6 (37.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2.625	0.269	NS
	Sensitive	0 (0.0%)	0 (0.0%)	10 (62.5%)	0 (0.0%)	1 (100.0%)	4 (100.0%)			
	Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
CAZ	Resistant	20 (66.7%)	4 (57.1%)	18 (90.0%)	4 (100.0%)	5 (100.0%)	14 (93.3%)	10.884	0.054	NS
	Sensitive	10 (33.3%)	3 (42.9%)	2 (10.0%)	0 (0.0%)	0 (0.0%)	1 (6.7%)			
	Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
CRO	Resistant	24 (80.0%)	5 (71.4%)	16 (80.0%)	4 (100.0%)	1 (20.0%)	10 (66.7%)	10.381	0.065	NS
	Sensitive	6 (20.0%)	2 (28.6%)	4 (20.0%)	0 (0.0%)	4 (80.0%)	5 (33.3%)			
	Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
FEP	Resistant	25 (83.3%)	5 (71.4%)	18 (90.0%)	4 (100.0%)	4 (80.0%)	7 (46.7%)	11.985	0.035	S
	Sensitive	5 (16.7%)	2 (28.6%)	2 (10.0%)	0 (0.0%)	1 (20.0%)	8 (53.3%)			
	Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
IPM	Resistant	9 (31.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	2 (40.0%)	3 (27.3%)	12.285	0.266	NS
	Sensitive	20 (69.0%)	4 (66.7%)	3 (100.0%)	4 (100.0%)	3 (60.0%)	8 (72.7%)			
	Intermediate	0 (0.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
MEM	Resistant	16 (53.3%)	4 (57.1%)	10 (50.0%)	3 (75.0%)	1 (20.0%)	9 (60.0%)	3.380	0.642	NS
	Sensitive	14 (46.7%)	3 (42.9%)	10 (50.0%)	1 (25.0%)	4 (80.0%)	6 (40.0%)			

	Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
AK	Resistant	23 (76.7%)	6 (85.7%)	17 (85.0%)	3 (75.0%)	5 (100.0%)	14 (93.3%)	11.362	0.330	NS
	Sensitive	6 (20.0%)	0 (0.0%)	1 (5.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
	Intermediate	1 (3.3%)	1 (14.3%)	2 (10.0%)	1 (25.0%)	0 (0.0%)	1 (6.7%)			
CN	Resistant	12 (40.0%)	5 (71.4%)	13 (65.0%)	3 (75.0%)	4 (80.0%)	12 (80.0%)	9.329	0.097	NS
	Sensitive	18 (60.0%)	2 (28.6%)	7 (35.0%)	1 (25.0%)	1 (20.0%)	3 (20.0%)			
	Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
SXT	Resistant	22 (73.3%)	5 (71.4%)	8 (40.0%)	2 (50.0%)	4 (80.0%)	11 (73.3%)	7.782	0.169	NS
	Sensitive	8 (26.7%)	2 (28.6%)	12 (60.0%)	2 (50.0%)	1 (20.0%)	4 (26.7%)			
	Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)			
ATM	Resistant	23 (76.7%)	4 (57.1%)	17 (85.0%)	3 (75.0%)	3 (60.0%)	13 (86.7%)	20.525	0.025	S
	Sensitive	7 (23.3%)	3 (42.9%)	3 (15.0%)	0 (0.0%)	1 (20.0%)	2 (13.3%)			
	Intermediate	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (25.0%)	1 (20.0%)	0 (0.0%)			
CIP	Resistant	23 (76.7%)	6 (85.7%)	17 (85.0%)	4 (100.0%)	3 (60.0%)	13 (86.7%)	5.738	0.837	NS
	Sensitive	5 (16.7%)	1 (14.3%)	2 (10.0%)	0 (0.0%)	2 (40.0%)	1 (6.7%)			
	Intermediate	2 (6.7%)	0 (0.0%)	1 (5.0%)	0 (0.0%)	0 (0.0%)	1 (6.7%)			

p-value > 0.05: Non significant; *p*-value < 0.05: Significant; *p*-value < 0.01: Highly significant. *: Chi-square test

Discussion

Burn wound infection represents one of the most usual and inevitable obstacles in the process of wound healing. In the present study, more females (54.2%) were enrolled in the study as compared to males (45.8%). This is similar to a study conducted in India by **Datta et al.** where more females (76.4 %) than males (23.6 %) were affected [12]. The explanation for this may be that females are more likely than males to be involved in everyday household tasks such as cooking in kitchens, where there is a higher risk of flame burns. In the present study, admitted patients had total burn surface area ranged from 9% to 40%, which was similar to total burn surface area in the studies done by **Saaq et al.** [13] and **Jauhari et al.** [14]. Scalding was the most frequent cause of burn injuries.

We discovered that 97.5 percent of the cultures were growing positively. This contrasted with the findings of a report conducted by **Chaudhary et al.** [3] that resulted in lower rate of infection 68.5%. And it is similar to a study conducted in India, which found that 93 % of the samples showed positive progress[15]. Within the first week in the hospital, positive cultures were slightly more common.

Pseudomonas aeruginosa was the frequent pathogen isolated from burn injuries in this study. This was supported by several reports [13, 16-18], this may be explained by that most of these studies were conducted in areas with moist weather which is suitable for growth of *Pseudomonas aeruginosa*.

Hegde and Bhandary [19] on the other hand, found that *Acinetobacter* was the most common organism infecting burn wounds. **Altparlak et al.** [20] found *Staphylococcus aureus* the most frequent organism. *Klebsiella pneumoniae* was found to be the most common causative organism (47.5 %) in another study done in Egypt by [21]. *Pseudomonas aeruginosa* was found to be sensitive to ceftazidime and meropenem in the study by **Datta et al.** with 55.6 percent of isolates susceptible to each antibiotic. In the current study, *Pseudomonas aeruginosa* was only 10.0 % sensitive to ampicillin, and 33.3 % sensitive to ceftazidime. Sensitivity to imipenem was (69.0%) and meropenem (46.7 %). This clearly shows that *Pseudomonas aeruginosa* has developed resistance to drugs that were previously thought to be effective. The poor sensitivity *Pseudomonas aeruginosa* necessitates the development of new antibiotics or other methods of controlling infection by this organism. In the present study, *Staphylococcus aureus* was found to be the second most common organism infecting burn wounds; however, it was the most common pathogen in another study conducted in India by **Datta et al.** [12]. Our findings were close to those of **Sharma et al.** [22] in India, who found *Staphylococcus aureus* to be the second most common organism in burn wounds. Gram-positive organisms are the first organisms that colonize the burn injury in the first 48 hours because they can survive in hair follicles and sweat glands, and surface antibiotics are the only way to eradicate

these Gram positive organisms. Most of *Staphylococcus aureus* isolates in the current study were sensitive to vancomycin (58.8%) and linezolid (62.5%) thus it can be given as empirical therapy for the control of MRSA infections.

Klebsiella pneumoniae were the third most common isolate in this work, it represented 15.7% of all isolates, however *Klebsiella* was found to be the second most common organism in the report done by Saaiq et al. [13]. In the present study *Klebsiella pneumoniae* were most sensitive to imipenem (66.7%) although piperacillin and tazobactam were found to be the most effective against *Klebsiella pneumoniae* isolates (80.95%) in the study by Saaiq et al. [13].

Proteus vulgaris and *Enterococcus faecalis* were found to be the third most common species infecting burn wounds with percentage of 6.7% for each of them, This is close to a study by Datta et al. [12]. In the present study, *Proteus vulgaris* was found in 3.33% of the cultures, while in the study by Lakshmi et al. [15] *Proteus vulgaris* represented in 17.4% of the cultures. In the research conducted by Hegde et al. [19] the percentage of *Enterococcus faecalis* was 4%.

Escherichia coli (4.8%), *Acinetobacter baumannii* (3.6%), and *Micrococcus leuteus* (1.2%) were the least common pathogens in the current study. In comparison to a study in India by Lakshmi et al., [15], *E. coli* was found to be the second most common organism infecting burn wounds. According to a report by Chaudhary et al. [3]. *Acinetobacter baumannii* was the third pathogen among the organisms infecting burn wounds.

The most common bacterial combination in the present study (20.59%) was *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*; however, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were the most common bacterial combination (12.9%) in the study by Rajbahak et al. [23].

Significant growth was observed in 81 (97.5%) of the 83 wound swab samples analysed on plates of conventional agar and Hichrome universal agar; additionally, 10 (12.04%) and 15 (18.1%) plates showed mixed growth on conventional and Hichrome universal agar, respectively, with no growth observed in 2 (2.4%) plates on both. Out of 15 (100%) polymicrobial growths 15(100%) were demonstrated distinctly on Hichrome Universal agar and only 10 (66.67%) were obtained from other agar media. An analysis of the number of samples

containing a mixed bacterial population revealed that the chromogenic media used in this study allowed for rapid pathogen pre-differentiation after 24 hours, rather than the 48 hours needed by conventional media. It is difficult to interpret mixed cultures of burn wounds. Many biochemical tests should be performed to validate the diagnosis of mixed wound swab cultures [24], As a result, chromogenic media can make detecting polymicrobial growth easier. It allows for the precise identification of common species, reducing the process's turn-around- time and cost [25].

Hence, using chromogenic media for conclusive identification of often isolated species and probable identification of less frequently isolated organisms, along with simple biochemical test for their species confirmation is a viable option [24].

Conclusion

The most common pathogens isolated in this study was *Pseudomonas aeruginosa*. Antibiotics such as meropenem and aminoglycosides have been found to be the most sensitive against Gram negative bacteria, whereas vancomycin and linezolid are the most effective against Gram positive bacteria. Chromogenic media is a practical choice for identifying the frequently isolated organisms and reducing the overall time and cost of the procedure.

Recommendations

1. Regular antimicrobial surveillance of Ain Shams University hospitals should be promoted to guide empiric choice of antimicrobials for burn treatment.
2. Further studies are recommended in other burn centers in order to elicit the corresponding results to help them in the management of burn wound infections.
3. Close supervision of children in their early stages of development to ward off any burn occurrences.
4. Use of chromogenic media for rapid identification of organisms and save more lives from complications of burn that result from delay of identification of organisms.

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Conflict of interest

There has been no funding for this research, and there is no competing personal financial interests in relation to the work described. Each author mentioned in the manuscript had seen and accepted the submission of this version of the manuscript and accepts full responsibility for it.

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