SHORT COMMUNICATION

Fungal isolation from wound samples submitted for culture at a tertiary care hospital laboratory

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Abstract

Fungal wound infections are increasing worldwide. The aim of this retrospective study, conducted at the Aga Khan University laboratory, Karachi, Pakistan, was to determine the frequency of fungal isolation in wound specimens. Data of wound samples received for culture from all over the country between September and October 2018 was reviewed. Samples were processed for bacterial cultures and additionally inoculated on Sabouraud's dextrose medium. Demographic information, medical history and information on the type of wound was collected. A total of 140 cases were included, of which 87 (81%) were culture positive, while 10 (7%) cases yielded fungi. Burn and blast wounds had the highest proportion of fungal isolation, i.e. 2 out of 4 (50%). Candida species were the most common fungi (n=4), followed by Fusarium species (n=3). This study reports an alarming rate of fungal wound infections. As fungal necrotising wound infections have high morbidity and mortality, it is, therefore, important to accurately diagnose and treat such infections in local setting.

Keywords: Wounds and Injuries, Fungal Infections, Diabetic Foot, Infections, Skin and Soft Tissue Infections.

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Introduction

Wounds may be secondary to skin and soft tissue infections (SSTI), diabetes, burns, trauma, or surgical interventions. Even though bacteria remain the most common pathogen isolated from wounds, the occurrence of fungal infections is increasing.[1,2] Studies report that patients who were administered systemic antibiotics had significantly higher fungal diversity.[3] Moreover, wound deterioration was also linked with an increase in fungal diversity which is most likely secondary to advanced bacterial infection and administration of more antibiotics.[3] Studies have reported the prevalence of fungal isolates to be as high as 22% in chronic wounds,[2] 71% in diabetic foot ulcer,[3] 8-12% in trauma patients,[4] and 5-7% in combat trauma-related wounds.[5]

Post-traumatic fungal infections, although rare, can be lethal for patients. Due to non-specific clinical features, these cases are often misdiagnosed causing delays in antifungal treatment.[4,5] The recognition of fungal isolates is also important to choose the correct treatment for bacteria in co-infections as wounds often develop bacterial and fungal biofilms with fungi providing a scaffold for bacterial attachment and offer protection and resistance against antibiotics.[3] This bacterial-fungal interaction has serious implications leading to delayed wound healing.

Rationale of the study: Despite a high population of at-risk patients, no study has reported the frequency of fungal isolation in wounds in Pakistan. This knowledge is required to initiate prompt management of patients with wound infections.

The objective of this study was therefore to report the frequency of fungal isolation in wound infection samples.

Methods

The study was conducted at the Aga Khan University Hospital Karachi (AKUH) which has a College of American Pathologists accredited laboratory with >250 peripheral collection centres.

The AKUH laboratory receives samples from all over Pakistan, but mostly from hospitals within Karachi and other cities of Sindh. A comparatively lower number of specimens are received from Punjab, Khyber Pakhtunkhwa, and Baluchistan.

All tissue and pus samples received for bacteriological assessment at AKUH laboratory from September to October 2018 were included in the study. Therefore, the sampling technique was consecutive sampling. These specimens were sent to the laboratory at the discretion of the referring physician and the researchers had no control over these referrals.

Inclusion criteria: All pus, tissue, and swabs from wounds that came to the AKUH laboratory for aerobic and anaerobic culture were included.

Exclusion criteria: Sterile fluids, deep tissues from

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cavities, brain and bones were excluded. Duplicate samples (more than one sample received from the same patient) during the study period were excluded.

Specimen processing: All samples were processed according to the laboratory standards modified to include more selective media for mixed cultures.[6] Samples were inoculated on chocolate and sheep blood agar with Colistin and Nalidixic acid incubated in 5% CO2; a triplate with MacConkey, mannitol salt and Sabouraud's dextrose agar (Figure) incubated in ambient environment; and sheep blood agar incubated anaerobically. All cases were enriched by inoculating in cooked meat broth.

Clinical information recorded at the time of reporting was collected. Variables such as patient's age, gender, location, type of wound, and culture result were collected. The data was entered in IBM SPSS software version 23 and descriptive analysis was done.

Exemption was granted from the institutional Ethical Review Committee (No: 2020-4854-10990).

Operational definitions: Skin and soft tissue infections: Specimens from patients with infection of skin and underlying soft tissues, fascia, and muscles. Wounds that were not acquired in the hospital, nor associated with diabetes and without history of trauma were classified as miscellaneous SSTI (mSSTI).

Nosocomial wound infection: Specimens from surgical site infections and wounds acquired in the hospital due to medical intervention.

Figure: Petri dishes of (i) triplate with MacConkey Agar, Mannitol Salt Agar and Sabouraud's Dextrose Agar (top) (ii) Chocolate Agar (bottom right) and (iii) Sheep Blood Agar (bottom left).

Traumatic wounds: Specimens from patients with injuries due to road traffic accidents, violence or fractures and household accidents.

Diabetic wounds: Specimens from lesions with presence of inflammation or purulence in diabetic patients.

Burns and blast wounds: Specimens from patients with electrical, chemical or thermal burns or bomb blast injuries.

Unknown type: Specimens unaccompanied with history.

Results

A total of 140 patients with wound infections were identified. The median age of the patients was 30.5 years (IQR: 33.5; range 27 days-87 years). Thirty (21.4%) patients were <18 years and 15 (10.7%) were ≥65 years old. Sixty-eight (48.6%) patients were females and 72 (51.4%) were males. Of the total, 63 (44.4%) samples were from Karachi, 26 (18.3%) from elsewhere in Sindh, 37 (26%) from Punjab, and 16 (11.3%) from Quetta and Khyber Pakhtunkhwa.

The most common category of wound was mSSTI, i.e. 80 out of 140 (57%), followed by nosocomial 21 (15%), traumatic wounds 12 (8.5%), and diabetic wounds 11 (8%). Of the total 140 samples, 107 (76.4%) were culture positive, 87 (62.1%) samples yielded pathogens, and 20 (14.2%) samples grew commensals and skin flora (Table-1).

Overall, bacteria were the most isolated pathogen [85 out of 140 (60.7%)], followed by fungi [10 out of 140 (7.1%)]. There was a mixed growth of bacteria and fungi in 8 out of 140 (5.7%) cases. Samples from burn and blast lesions [2 out of 4 (50%)], followed by traumatic wounds [1 of 8 (8%)] had the highest percentage of mixed growth. Burns and blast wounds [2 of 4 (50%)] had the highest proportion of fungal isolates, followed by lesions of unknown origin [2 of 12 (17%)], and traumatic wounds [1

Table-1: Distribution of type of skin and soft tissue infections, culture positivity and isolated pathogens in all wound types.

Lesions	N	Culture positive (n)	Pathogen isolated (n)	Bacteria [n (%)]	Fungi [n (%)]
Skin and soft tissue infections	80	60	51	51(64)	4 (5)
Nosocomial wound infection	22	19	14	14 (63)	1 (4.5)
Traumatic wounds	12	8	7	7 (58)	1 (8)
Unknown type	12	9	8	6 (50)	2 (17)
Diabetic wounds	11	8	3	3 (27)	0
Burns and blast wounds	4	4	4	4 (100)	2 (50)
Total	140	107	87	85 (61)	10 (7)

Table-2: Distribution of fungal isolates and wound categories.

Fungal type	Total	SSTI	Diabetic Wounds	Burns and Blast	Traumatic	Nosocomial	Unknown
Candida spp.	4	3	0	0	0	0	1
Aspergillus spp.	2	1	0	0	0	0	1
Fusarium spp.	3	0	0	2	0	1	0
Bipolaris spp.	2	0	0	0	1	0	1
Curvularia spp.	1	0	0	0	0	0	1
Total	12*	4	0	2	1	1	4

*There was a total of 10 cases of fungal isolation. 9 cases had single type of fungi and one case had three fungi including Candida, Aspergillus and Bipolaris.
SSTI: Skin and soft tissue infections.

of 12 (8%)] (Table-2).

Details of fungal isolates is shown in Table-2. The most common fungal pathogen was Candida species [4 out of 10 (40%)], followed by Fusarium spp [3 out of 10 (30%)]. A total of 10 cases showed fungal isolation, of which 9 cases had single type of fungi and one case had three fungi i.e. Candida, Aspergillus and Bipolaris.

Discussion

The current study reports an alarming rate of fungal isolation [10/140 (7%)] in wound specimens. A similar study from India also reported fungal isolation in 37 out of 288 (12.8%) pus and tissue samples, of which 24 were yeast and 13 were moulds.[7] Burns and blast wounds had the highest proportion of fungal isolates [2 out of 4 (50%)] followed by traumatic wounds [1 of 12 (8%)] in current study. A high rate of fungal isolation in burn patients [27 of 70 (39%)] has also been reported from Pakistan which is consistent with global data and the current study.[8] Similarly, fungal infections in blast and other accidental trauma are emerging as serious complications leading to high morbidity and mortality.[4,5] Fungi are prevalent in the environment, leading to contamination of open wound after trauma, especially after road traffic accidents, burns, blast injury, and other disasters.

Fungi are also prevalent in surgical site infections and other nosocomial SSTIs. Surface and environmental contamination with fungi was reported to be a significant factor in the development of surgical site infections in 1,267 patients.[9]

Fungi are a leading cause of necrotising wound infections and are a significant cause of morbidity and mortality, as reported in a recent study.[10] Therefore, accurate detection of fungi from wound is extremely important to improve the outcomes. Often, culture-dependent methods may underestimate fungal diversity and bioburden. A study using sequencing, a non-culture-based detection method detected fungi in 80% of the 100 cases of diabetic foot wound as compared to only 5% in culture.[3] In our study, diabetic wounds did not have high fungal isolation. This may be due to the small sample size, short study duration and reliance on culture-based methods only.

The limitations of this study are a small sample size, single centre, short study period and use of culture-based technique only. However, in resource limited settings where non-culture-based methods such as sequencing are expensive, optimisation of culture using fungal selective media may increase the yield of fungi.[8]

Conclusion

The study reports an alarmingly high rate of fungal wound infections. As fungal necrotising wound infections have high morbidity and mortality, it is, therefore, important to accurately diagnose and treat such infections in local settings.

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