ORIGINAL ARTICLE

Bacteriological Profile of Wound Swab and Pus Samples Using Conventional Media and Chromogenic Medium

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Abstract:

Background: Wound infections continue to be a cause of concern as they can delay healing and cause wound breakdown. Their effective treatment demands quick isolation and identification of causative organisms with appropriate antibiotic sensitivity pattern. *Material and Methods*: Wound swab and pus samples received from inpatient as well as outpatient department of all age groups and both genders were processed using conventional media as well as chromogenic medium (HiCrome UTI) and results of both were compared. Antibiotic sensitivity testing was done on Vitek 2 Compact automated system. *Results*: Among 342 samples, 77% showed growth. Fifty eight percentage were Gram negative and 42% were Gram positive organisms. Polymicrobial growth was seen in 11% of samples. HiCrome UTI isolated all organisms in culture. Colony characteristics and colour of all isolates on HiCrome UTI were comparable to their identification on Vitek 2 Compact. Among the Gram-positive organisms, commonest was Methicillin Sensitive *Staphylococcus aureus* (MSSA 42%) followed by Methicillin Resistant *Staphylococcus aureus* (MRSA 33%), *Enterococcus faecalis* (10%), *Staphylococcus epidermidis* (8%), *Staphylococcus haemolyticus* (3%), *Streptococcus pyogenes* (2%) and *Streptococcus agalactiae* (2%). Most of the Gram positive organisms were sensitive to vancomycin, teicoplanin, linezolid and clindamycin The common Gram negative organisms were *E. coli* (36%), *Klebsiella pneumoniae* (20%), *Pseudomonas aeruginosa* (18%), *Proteus mirabilis* (7%), *Enterobacter cloacae* (6%) and *Acinetobacter baumannii* (4%). Most of the Gram negative organisms were sensitive to cefepime, beta lactams-beta lactamase inhibitors, aminoglycosides and fluoro-quinolones. *Conclusion*: Gram-negative organisms predominated in our study. HiCrome UTI agar can be used as a cost-effective approach for rapid isolation of all organisms. It gives definite identification of common organisms and thus reduces turn-around-time for the same. It provides presumptive identification of infrequent organisms which can be further confirmed by simple biochemical tests. Hence these properties of HiCrome UTI agar help serve the purpose especially from mixed cultures and in resource constraint settings.

Keywords: Bacteriological Profile, Chromogenic Medium, Pus, Wound Swabs

Introduction:

Wound is defined as a breakdown in the protective function of the skin and loss of continuity of epithelium with or without loss of underlying connective tissue [1]. A variety of microorganisms can lead to wound infection. It is also one of the most common health-care associated infection associated with longer hospital stay and increased cost of healthcare [1]. Diabetes is a common co-morbidity leading to wound infections [2]. Also, diabetic wound infections tend to be polymicrobial with significantly higher bacterial count and increased chances of colonisation with antimicro-

bial resistant organisms as compared to non-diabetics [2-3]. Hence, there is a distinct need for rapid identification of these organisms which further helps in starting empirical antibiotic treatment. Several available chromogenic media allow the identification of organisms based on distinctive colour patterns. Chromogenic medium uses chromogenic substrate which is hydrolysed to release a coloured product that remains highly localised on microbial colonies. This allows clear differentiation of microbes producing the target enzyme from those that do not and produces coloured colonies especially from mixed cultures. It thus helps in superior and early dentification of organisms, decreases the number of identification tests and saves manpower [4]. HiCrome UTI agar is one such chromogenic medium. However, its suitability for use with clinical specimens has been assessed only with urine samples and hardly any studies are done using other samples. This study was aimed at determining the bacteriological profile of infected wounds and pus samples using conventional and HiCrome UTI agar for isolation and identification of concerned pathogens and comparison of results on both along with antibiotic sensitivity testing.

Material and Methods:

The present study is an observational prospective study carried out in Department of Microbiology during the period of August 2019 to April 2020. The study was approved by the Scientific and Ethics Committee of the Institute. Three hundred and forty-two samples consisting of wound swabs and pus from patients of all age groups and both genders from Out-Patient Department (OPD) and In-Patient Department (IPD) received in microbiology laboratory were included in the study. Of these, wound swabs from various body sites were 146, 30 from infected operation sites and 21 from infected sebaceous cyst. Pus samples from sites like ear, back, breast, eye and adnexa, furuncle pustules were 58, 52 from abscesses like inguinal, gluteal, perianal, thigh, abdominal, chest and scrotal, 25 from paronychia and 10 from internal organs like appendix, endometrium and gall bladder. Gram stain was done from all samples. In case of wound swabs, two swabs were obtained of which one was used for Gram stain. Samples were inoculated on 5% Sheep Blood agar, MacConkey agar and HiCrome UTI agar (Himedia laboratories, Mumbai) and incubated aerobically at 37^0 C for 24 hours and the plates were examined for growth [5]. From growth on conventional media, Gram stain was done and identification and antibiotic sensitivity test was done on Vitek 2 Compact automated system (Biomeriux, India). GP-ID card and AST 628/ST 03 card were used for Gram positive organisms and GN-ID card and AST280/281 for Gram negative organisms. Simultaneously, colour of the colony of organism on HiCrome UTI was noted. This was then compared with the identification given by Vitek 2 Compact automated system. Also, number and type of organisms and time required for identification by both methods was noted. *Staphylococcus aureus* ATCC 25923 and *E. coli* ATCC 25922 were used for purpose of quality control. Colony characteristics on HiCrome UTI after an incubation at 35-37^0C for 18-24 hours are: *E. coli* ATCC 25922- pink-purple colonies,

Klebsiella pneumoniae ATCC 13883-blue to purple mucoid colonies, *Pseudomonas aeruginosa* ATCC 27853– colourless (greenish pigment may be observed), *Proteus mirabilis* ATCC 12453-light brown, *Staphylococcus aureus* ATCC 25923-golden yellow, *Enterococcus faecalis* ATCC 29212- blue small [6].

Statistical Analysis:

Data was analysed using SPSS (Statistical Package for Social Science) program version 21 and results are presented as percentages of the group.

Results:

A total of three hundred and forty-two samples comprising wound swabs and pus were processed. Samples from age group below 18 years, 19-65 years and above 65 years were 25 (7%), 206 (60%) and 111 (33%) respectively. One hundred eighty samples were from males (53%) and 162 (47%) from females. One hundred fifty-seven (46%) samples were from IPD and 185 (54%) were from OPD. One hundred twenty six (37%) patients had diabetes mellitus. Two hundred twenty six (77%) samples showed growth and 80 (23%) did not show growth. Among the 291 organisms isolated, 169 (58%) were Gram negative and 122 (42%) were Gram positive (Fig.1). Polymicrobial infection was seen in 29 (11%) samples. Among the Gram positive organisms, Methicillin Sensitive *Staphylococcus aureus* (MSSA) were 52 (42%), Methicillin Resistant *Staphylococcus aureus* (MRSA) 40 (33%), *Enterococcus faecalis* 12 (10%) *Staphylococcus epidermidis* 10 (8%), *Staphylococcus haemolyticus* 3 (3%), *Streptococcus pyogenes* 3 (2%) and *Streptococcus agalactiae* 2 (2%) (Fig.2). Most of the Gram positive organisms were sensitive to vancomycin, teicoplanin, linezolid, clindamycin and tetracycline and showed resistance to penicillin, amoxycillin-clavulanic acid, erythromycin and ciprofloxacin (Table 1). Among the Gram negative organisms, *E.coli* were 60 (36%), *Klebsiella pneumoniae* 34 (20%), *Pseudomonas aeruginosa* 31 (18%), *Proteus mirabilis* 11 (7%), *Enterobacter cloacae* 10 (6%), *Acinetobacter baumannii* 6 (4%), *Proteus vulgaris* 5(3%), *Morganella morganii* 5 (3%), *Citrobacter koseri* 5 (2%) and *Providencia rettgeri* 2 (1%) (Fig. 3) Most of the Gram negative isolates were sensitive to piperacillin-tazobactam, cefoperazone-sulbactam, cefepime, amikacin, gentamicin, ciprofloxacin, levofloxacin and sulfamethoprim-trimethoxazole and resistant to amoxycillin-clavulanic acid and third generation cephalosporins (Table 2). HiCrome UTI agar isolated all organisms without the need for subculture. Colony characteristics of *E. coli*, *K. pneumoniae*, *P. aeruginosa, S. aureus* and *E. faecalis* were comparable to their identification on Vitek 2 Compact system. On HiCrome UTI, colonies of *Enterobacter and Citrobacter* were light blue. Coagulase negative *Staphylococcus* (CONS-*S. epidermidis* and *S. haemolyticus*) were white. *Acinetobacter baumannii* were white mucoid. *Proteus species*, *Morganella morganii* and *Providencia rettgeri* were light brown and those of *Streptococcus* were pinpoint colourless (Fig.4).

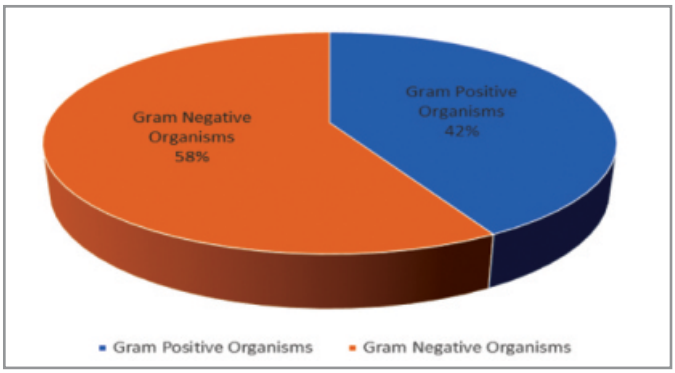

Fig. 1: Organisms Isolated

Table 1: Antibiotic Sensitivity of Gram Positive Organisms

	MSSA 52 (42%)	**MRSA 40 (33%)**	***E. faecalis* 12 (10%)**	***S. epidermidis* 10 (8%)**	***S. haemolyticus* 3 (3%)**	***S. pyogenes* 3 (2%)**	***S. agalactiae* 2(2%)**
Penicillin	38%	0	0	30%	0	100%	100%
Cloxacillin	35%	0	0	30%	66%	100%	100%
Amoxy-clav	42%	0	33%	0	33%	100%	100%
Cefazolin	90%	0	0%	60%	100%	100%	100%
Cefuroxime	92%	0	0%	60%	100%	100%	100%
Gentamicin	94%	65%	0	70%	66%	100%	50%
Erythromycin	58%	55%	08%	30%	0	100%	50%
Clindamycin	80%	58%	0	50%	100%	100%	50%
Co-trimoxazole	48%	63%	0	30%	33%	0	50%
Tetracycline	92%	90%	67%	58%	100%	0	100%
Ciprofloxacin	42%	48%	56%	42%	66%	100%	50%
Levofloxacin	31%	45%	56%	42%	66%	100%	50%
Vancomycin	100%	100%	100%	100%	100%	100%	100%
Teicoplanin	100%	100%	100%	100%	100%	100%	100%
Linezolid	100%	100%	100%	100%	100%	100%	100%

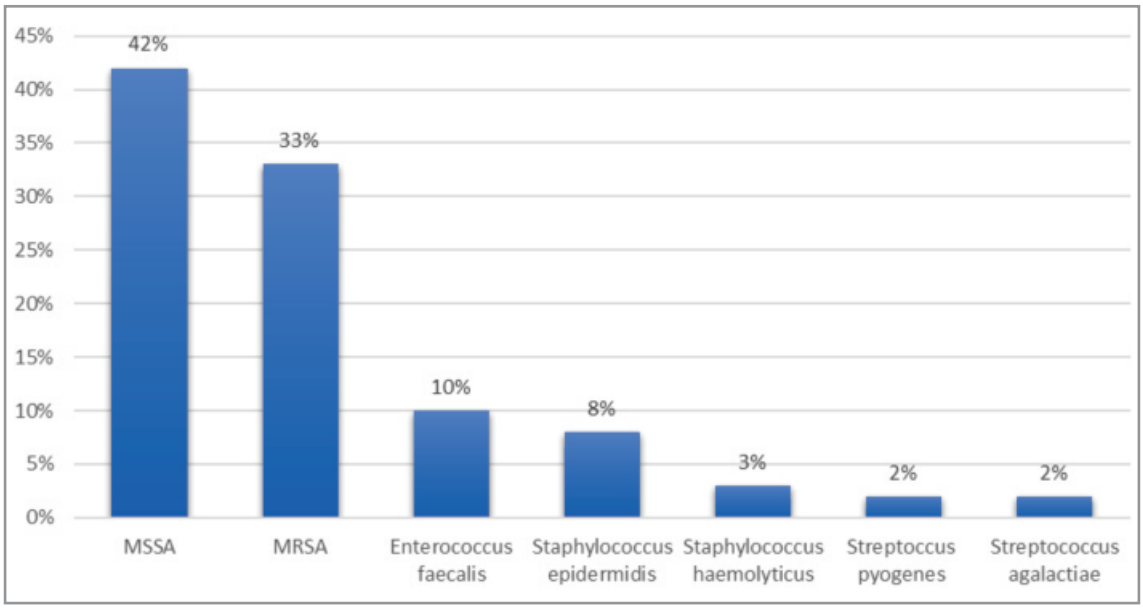

Fig. 2: Gram Positive Organisms Isolated

Table 2: Antibiotic Sensitivity of Gram Negative Organisms

Antibiotics	***E. coli*** **60 (36%)**	***K pneumonie*** **34 (20%)**	***P. aeruginosa*** **31 (18%)**	***P mirabilis*** **11 (7%)**	***E cloacae*** **10 (6%)**	***A baumannii*** **6 (4%)**	***P. vulgaris*** **5 (3%)**	***M. morganii*** **5 (3%)**	***C. koseri*** **5 (2%)**	***P. rettgeri*** **2 (1%)**
Ampicillin	10	0	0	55	20	0	60	100	0	0
Amoxycillin-clavulanate	33	21	0	45	10	0	60	0	75	0
Cefotaxime	27	52	0	55	50	0	100	40	75	50
Ceftriaxone	28	52	16	55	60	0	100	40	100	100
Cefepime	75	67	71	73	80	0	100	80	100	100
Amikacin	97	73	90	82	90	0	100	100	100	100
Gentamicin	85	73	97	64	80	33	100	100	100	100
Co-trimoxazole	50	55	32	55	60	50	60	60	100	50
Ciprofloxacin	52	70	74	73	90	33	100	100	100	100
Levofloxacin	57	70	71	73	80	50	100	100	100	100
Piperacillin-tazobactam	83	64	71	64	80	0	100	100	100	100
Cefoperazone-sulbactum	82	64	81	64	90	0	100	100	75	100

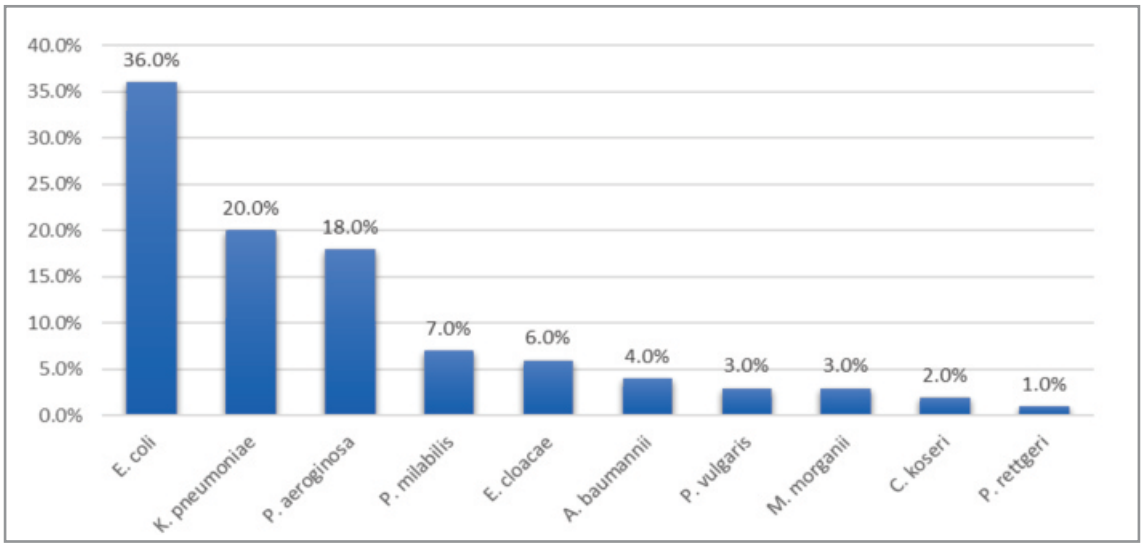

Fig. 3: Gram Negative Organisms Isolated

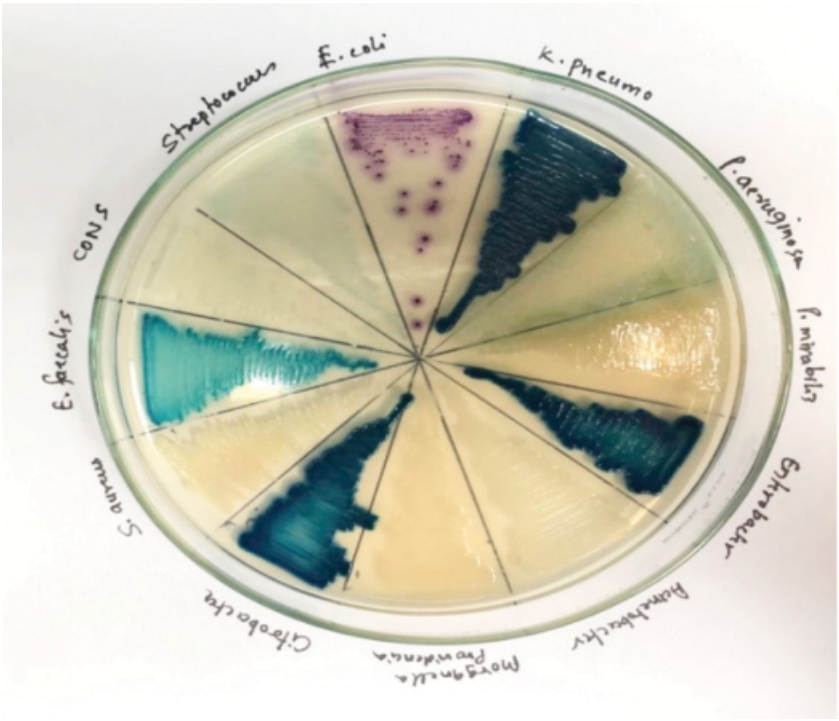

Fig. 4: Growth of Organisms on HiCrome UTI Agar

Discussion:

Wounds occur due to loss of intact skin due to damage caused by various external forces like surgical wounds, burns, bites, abrasions, cuts, or severe traumatic wounds. Colonisation of the wound by bacterial flora followed by their proliferation at wound site may lead to wound infection. Immune cells come in that area as a result of body's defence mechanism to fight the bacteria. Accumulation of these cells produces a thick liquid called pus [7]. Wound infections prolong duration of hospital stay as compared to healthy wounds [8]. The bacterial profile of pus samples remains same overall but antibiotic resistance pattern varies [7]. In our study, 53% samples were from males and 47% from females which was comparable to that of Bankar *et al*. where samples from males and females were 60% and 40% respectively [7]. Nishanthy *et al*. in their study found samples from males and females to be 66% and 34% respectively [8]. Seventy-seven percentage of our samples showed growth similar to study by Nithya *et al.* where positivity rate was 78.55% [9]. Majority of our samples were from 19-65 years age group alike findings of Sudhaharan *et al*. in which the median age of patients was 47 years [10]. In our samples, Gram negative organisms (58%) predominated Gram positive organisms (42%). This was analogous to other such studies by Mahat *et al*. and Yakha *et al*. where there was similar organism preponderance among such samples [11-12]. Commonest Gram-positive organism in our study was *Staphylococcus aureus* followed by *Enterococcus faecalis,* CONS and *Streptococcus species*. Amongst the Gram negatives, *E. coli* was the prime organism followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Proteus mirabilis, Acinetobacter baumannii, Proteus vulgaris, Enterobacter cloacae* and *Citrobacter koseri.* Various studies by Chaudhary *et al.* [13], Sudhaharan *et al.* [10], Bankar *et al.* [7] and Nishanty *et al.* [8] showed similar bacteriological profile of their samples. Most of our isolates of *E. coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were sensitive to cefepime, piperacillin-tazobactam, cefoperazone-sulbactam, quinolones and aminoglycosides. Isolates of *Proteus, Enterobacter* and *Citrobacter* were sensitive to most of the antibiotics. This pattern was in accordance with findings of Wadekar *et al.* [14] and Trojan *et al*. [15]. *Acinetobacter* isolates from our samples were multidrug resistant alike other studies [10, 15]. Among the Gram positive organisms all were sensitive to vancomycin, teicoplanin, linezolid and clindamycin comparable to other studies [7, 14]. *MSSA* (42%) isolates were more than *MRSA* (33%) as seen in studies by Singh *et al.* [16], Shetty *et al.* [17] and Sudhaharan *et al*. [10]. In diabetic patients, polymicrobial wound infections are common and many such wounds are colonised with multidrug resistant organisms [3]. In our study, 126 patients had diabetes mellitus. Samples of 29 (11%) patients showed polymicrobial growth of which 14 patients had diabetes mellitus alike finding of Sudhaharn *et al.* where combined infections were seen in 7% patients [10]. All our isolates grew on HiCrome UTI agar. HiCrome UTI made it easier to pick up polymicrobial growth from mixed cultures as compared to conventional media. It thus reduces time and cost of culture by decreasing use of media and need for sub-culturing. Colony characteristics of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*

and *E. faecalis* were comparable to their identification on Vitek 2 Compact. These were also the commonest organisms isolated in our study. Other infrequent organisms were tentatively identified on HiCrome UTI agar which could be further confirmed by simple biochemical tests. Similar inference was made by Ohkusu in his study of organisms that chromogenic media along with simple biochemical tests can be used to identify isolates from various samples [18]. Similar observation regarding chromogenic medium was also made by Watanable *et al*. [19]. Hence, chromogenic media for definitive identification of frequently isolated organisms and probable identification of less frequent organisms along with simple biochemical test for their species confirmation is a workable option.

Conclusion:

This study emphasizes various organisms isolated from pus and wound infections and their antibiotic sensitivity pattern which is beneficial in empirical treatment of patients with such infections. HiCrome UTI facilitates growth of all organisms from pus and wound swabs and provides definitive and presumptive identification of common and uncommon organisms respectively, especially from mixed cultures and is thus time saving and economical. Species confirmation of the uncommon organisms can be further done using additional simple biochemical tests. Thus, HiCrome UTI along with adjunctive simple biochemical test is a practical and handy option for such samples especially in fiscal constraint settings.

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