Original Article

Bacterial Profile with Antimicrobial Sensitivity Pattern of Different Pyogenic Infections Treated in a Tertiary Care Hospital at Kolkata

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Background : It is pertinent that in order to mitigate the burden and its associated complications of pyogenic infections, a robust antimicrobial therapy is the need of the millennium. The world is very badly hit by the recent era of antibiotic resistance. This has posed an impediment to the treatment options which has been much been curtailed.

Objective : To identify the spectrum of causative organisms from pus cell, to find out pattern of antibiotic susceptibility of most predominant microbial agents. Methodology: A descriptive Cross-sectional study was carried out from 26th July 2016-25th July 2018 in the Department of Microbiology, Calcutta National Medical College, Kolkata involving all the 90 and 370 specimens of Pus and Wound swab collected via recommended procedure from the patients attending OPD and admitted in IPD and sent for culture and sensitivity testing. Specimen belonged to post-surgical complicated cases were 140 and rest of the samples were not related to surgery. Results: Culture was positive in 54.12% with slight dominance of male gender and in 21-40 years age group. Maximum comprised of Staphylococcus aureus species of organisms (43.48%) which showed high sensitivity to the drugs Erythromycin, Vancomycin, Doxycycline with one fourth Methicillin resistant strain. The second predominant organism was the gram-negative species Klebsiella sp. (23.91%) found to have maximum sensitivity to Colistin, Imipenem, Amikacin, Levofloxacin and Gentamicin.

Conclusion : In clinical practice Pyogenic skin infections are mainly resistant to one or more of the antibiotics, thereby limiting the treatment options. It is pertinent to have an antibiotic policy of health facilities for treatment of patients and reducing resistance.

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Key words **: Pyogenic infection, Bacteria, Antimicrobial, Sensitivity.**

Those so-called Pyogenic infections are usually characterized by local inflammations, the cause of which has been attributed to be due to pyogenic bacteria that can produce the accumulation of dead leukocytes and infectious agents commonly known as pus. Such infections of the human skin and soft tissue infections are caused during or after trauma, burn injuries and surgical procedures resulting in production of pus[2]. One of the most common causes of health care infection is the Surgical site infection with a reported rate of 2 to 20%[3]. A team led by World Health Organization researchers found developing countries carry much higher infection rates than the developed world and it is said "poor nation face: greater hospital infection burden"[4]. In India, the wound sepsis ranges from 10% to 33% in its occurrence[5,6].

Wound infections are contributed both by aerobic and anaerobic bacteria leading to significant morbidity, prolonged hospitalization which have great economic

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Editor's Comment :

- **Antibiotic Resistance has been a great challenge over the years. This has been aggravated by several factors like sell of OTC Drugs, Self-medication etc. In this new era of Emerging and Re-emerging infections, it is of utmost importance to be very vigilant on Antibiotic Policy.**
- **In this regard, every sample of Pyogenic Infection in tertiary care hospitals must be screened for bacterial profile with antimicrobial sensitivity pattern. this will not only reduce the burden of additional health costs, but can also help in developing a SOP of a robust antimicrobial therapy. Antibiotic stewardship is the call for the new Millennium.**

implication[7]. The emerging antibiotic resistance among pathogenic bacteria is viewed as serious threat to the public health worldwide. It has been observed that pus infections are mainly caused by Multidrug-resistant Gram-negative bacterial strains such as *E coli*, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Gram-positive methicillin-resistant Staphylococcus aureus (MRSA) and that too due to irrational prescribing habit[8-10]. As a result of emergence of multidrug-resistant bacteria, the treatment options have become limited in nature[10,11]. Few studies have shown a predictable bacterial profile and their antibiogram in certain areas[12]. Those clinicians who wants to initiate empirical treatment to his patients while laboratory culture reports are awaited can employ this strategy[13,14]. Thus our study mainly intended to develop a reliable data base about the bacterial profile.

Objectives :

1. To identify the spectrum of causative organisms of infection from the pus cell
2. To find out the pattern of antibiotic susceptibility of most predominant causative organisms

Materials and Methods

Our study is a descriptive Cross-sectional study which was undertaken in the Department of Microbiology, Calcutta National Medical College, for a period of over two years (26th July 2016- 25th July 2018) involving all 850 specimens of Pus and Wound swab which were sent for culture and sensitivity testing. Specimens collected from out-patient-department (OPD) were 90 in number and 370 were drawn from in-patient-department (IPD). From cases with post-operative complications 140 samples were collected and rest of the samples was not related to surgery of any kind.

Study tools and Reagents: Swab stick, sterile test tubes, Petri plates, inoculating loops, spirit lamps, cotton, incubator, microscope, glass slide and cover slip, blood agar, MacConkey agar, Mueller Hinton agar, Gram stain reagents, antimicrobial discs.

Control stains: Staphylococcus aureus ATCC 25922 and E. coli ATCC25923.

Specimen collection: A complete medical history with reference to onset, duration and progress of lesion and other relevant history was obtained. Then the specimens were collected by maintaining all aseptic measures, after cleaning the area around the lesion with 70% ethanol. Samples of pus were collected by a sterile swab stick or in sterile test tube. No sample was taken for anaerobic culture.

Culture of pus sample: Specimens were transported within two hours of collection. This was followed by processing on blood agar and MacConkey agar media by streaking method. Simultaneous gram staining was done directly from samples. His procedure was soon followed by incubating the culture plates at 37°C for 24 to 48 hours. The growth was noted from the colony and it was stained by gram staining. Following that, the biochemical tests were performed based on the organism.

Gram stain: The pus cells and the bacterial morphology, arrangement and the number of different types of organism were noted from direct stain. The colony morphology and strain from colony was correlated with previous direct stain.

Biochemical tests for gram positive bacteria were catalase, slide coagulase, tube coagulase and for gram negative isolates of test Indole, TSI, Citrate, Urease, Oxidase tests were performed routinely.

Using antimicrobial discs on Mueller Hinton agar applying the Kirby Bauer disc diffusion method (according to CLSI guideline), the sensitivity pattern was recorded.

Collected data were compiled in Micro soft excel and described by estimating various proportions. Displaying of data was achieved via tables and charts. The study was carried out after obtaining approval of the Institutional Ethics Committee.

Results

Total 850 specimens of pus were collected and processed with slight higher male preponderance (52.94%). Out of 850 pus samples, 460 (54.12%) showed growth with a male-female ratio of 1.09:1.0 (52.17% *versus* 47.83%). Around nine percent (8.69%) of the isolates were polyorganism. Majority of the culture positive participants belonged to the age group of 21-40 years closely followed by 41-60 years comprising of 43.48% and 34.78%, respectively (Table 1).

In this study gram positive organisms were found predominate with S (43.48%) on the top followed by gram negative Klebsiella species (23.91%).

Antibiotic susceptibility percentage: Analysis revealed that one fourth of the S aureus isolates showed resistance to Methicillin having sensitivity towards Linezolid and Vancomycin. The S aureus sensitive to Methicillin was also found to have good sensitivity to Doxicycline, Gentamycin, Levofloxacin and Vancomycin combination (Table 2).

In the study the most predominant species of organisms were found to be S aureus (43.48%) which showed high sensitivity to the drugs Erythromycin, Vancomycin, Doxicycline. (Table 3)

The second predominant organism was the gram negative species Klebsiella sp (23.91%) which was followed by Acinetobactor sp (13.04%) , Pseudomonas

Table1 — *Distribution of participants showing growth of organism as per age group and Gender (N=460)*

Age (Yr) Group	Male No (%)	Female No (%)	Total No (%)
1-20	30 (6.52)	50(10.87)	80(17.39)
21-40	90(19.57)	110(23.91)	200(43.48)
41-60	110(23.91)	50(10.87)	160 (34.78)
61-80	10(2.17)	10(2.17)	20(4.34)
Total	240(52.17)	220(47.83)	460 (100)

Table 2 — *Distribution of participants showing growth of Gram positive S aureus with its antibiotic sensitivity (n=200)*

Strain	Frequency (%)	Antibiotics to which Sensitive
S aureus (MRSA)	50 (25.0)	LZD, VAN
S aureus (MSSA)	150 (75.0)	DOX, GEN, LVX.VAN

MR/SSA= Methicillin resistant/sensitive S aureus,
LZD = Linezolid, VAN = Vancomycyn, DOX = Doxicycline,
GEN = Gentamycin, LVX=Levofloxacin,
LVX.VAN = combination of Levofloxacin & Vancomycin

aeroginosa (6.52%) and *E Coli* (4.35%). The gram-negative species were found to have maximum sensitivity to the drugs like Colistin, Imipenem, Amikacin, Levofloxacin and Gentamicin (Table 4).

Discussion

Age group :

Khanam RA *et al* observed in their study that around four out of every ten participants (42.0%) belonged to the age group of 20 to 40 years in concurrence to 43.48% in the present study[2]. From their study done at Kathmandu Razza MS *et al* also concluded that the maximum prevalence of the infection was prevalent in the age group 21-40 years[15].

Gender distribution :

The present study shows that males were found to be predominant (52.94%) as well as among the culture positives (52.17%) too. Khanam RA *et al* also reported higher proportion (56.1%) of male specimen[2]. Rao DVMVSVR *et al* observed that among the culture positive cases 58.82% were male[13]. Similar observations also made by Kamble P *et al* (67.0%), Mudassar S *et al* (64%), Mohammed A *et al* (59.10%), Muluye D *et al* (54.8%), Sudhaharan S *et al* and Khan I *et al* (59%)[16-21].

Culture positivity :

Analysis of the present study revealed culture positivity in 54.12% of all specimens and 8.69% showed polymicrobial growth. In a study by Sangwan J et al. that worked on 438 pus samples, about 72.6% of the culture showed positivity, surgical wards (39.7%) being the major contributor. Out of positive samples 82.3% were monomicrobial and 17.7% polymicrobial[1]. In majority (61.8%) of the cases aerobic culture was positive as observed by Khanam RA *et al*[2].

Kamble P *et al* reported growth in 92.0% of specimen out of which 85.87% cases showed monomicrobial[16]. In a study conducted by Rao DVMVSVR et al. about 89.47% of the cases yielded positive culture of which 95.09% was revealed to be pure bacterial isolates[13]. Sen M *et al* showed 59.38% samples to have single growth[22]. Kumari Pilli H P *et al* reported 21% culture positivity[23]. Shama M *et al* reported 73% culture positivity[24]. 83.9%. of the reported bacterial case was positive in a study by Mohammed A *et al*[18]. Muluye D *et al* found 70.2% culture positivity[19]. Another study by Sudhaharan S *et al* revealed that mono-microbial infections were found in 93.2% patients whereas combined infections with growth of two pathogens in 6.8%[20]. In their study Subha M *et al*[25]. observed 59.92% culture positivity having concurrence to the observation of growth in 61.11% of isolates made by Ghosh A *et al*[26].

Table 4 — *Distribution of participants according to antibiotic susceptibility of gram negative bacteria (GNB)*

Antibiotic[@]	Sensitivity of Bacteria to antibiotic (R:S)				
	Citrobacter koseri	Klebsiella sp.	E coli	Acineto-bacter sp.	Pseudomo-nassp.
AMC	100:0		100:0	—	—
LVX	—	—	—	—	100:0
AMK	0:100	57:43	50:50	75:25	0:100
CPM	—	—	—	—	100:0
CAZ	—	—	—	—	100:0
CTX	100:0	86:14	50:50	100:0	—
CTR	100:0	100:0	100:0	100:0	—
COT	—	57:43	100:0	75:25	—
CST	—	0:100	50:50	0:100	0:100
GEN	0:100	86:14	50:50	75:25	0:100
IMP	0:100	29:71	0:100	100:0	0:100
PTZ	100:0	57:43	0:100	50:50	100:0
PIB	—	—	—	—	0:100

[@]AMK =Amikacin, CPM = Cefexime, CAZ = Ceftazidine,
CTX = Cefotaxime, CST = Colistin, IMP = Imipenem,
PTZ = Piperacillin-tazobactum, PIB = Polymyxin B

Table 3 — *Distribution of participants according to antibiotic susceptibility of gram-positive bacteria (GPB)*

Organisms	Sensitivity of bacteria to antibiotics (R:S*)									
	GEN	HLGEN	CLIND	ERY	LVX	AMC	VAN	LZD	COT	DOX
S aureus	40:60	—	20:80	80:20	100: 0	40: 60	40:60	100: 0	90:10	20:80
Enterococcus	—	100: 0	100: 0	50:50	100: 0	100: 0	0:100	0:100	—	100: 0

R = Resistant, S = Sensitive, ERY = Erythromycin, LVX = levofloxacin, GEN = Gentamycin,
HLGEN = High level Gentamycin,CLIND = Clindamycin, AMC = Amoxicillin-Clavulanate,
COT = Cotrimoxazole

Isolates :

In our study, the most predominant species of organism was found to be S aureus (43.48%). Sangwan J *et al* observed S aureus to be the commonest isolate (24.2%) followed by Pseudomonas (21.4%), *E coli* (14.8%), Proteus spp (8.8%), Citrobacter spp (8.2%), Enterococcus (6.6%), Klebsiella spp (6.1%) and Streptococcus (2.2%). MRSA was 25.0% in the present study, compared to 48.9% found in another study[1]. Mudassar S et al. reported that among the culture positive pus samples S aureus accounted for 42%, P aeruginosa 19%, *E coli* 18%[17]. S aureus was found to the most predominant isolate (34%) followed by Klebsiella species (13%) in another study[18]. Muluye D and his associates found that majority (63.9%) were gram positive and around one third (36.1%) were gram negative. S aureus accounts 32.9% isolates, Coagulase Negative staphylococci [CONS] (14.7%), Streptococcus spp. (11.6%), Escherichia coli (9.5%), Klebsiella spp. (6.3%)[19]. From the observation of their study Shama M *et al* reported predominance (89%) of gram-negative isolates[24]. Predominance of S aureus was noted also by Subha M *et al* (26.32%) in their study[25]. Another study by Ghosh A *et al* showed that incidence of MRSA was half[26].

According to Khanam RA *et al* S aureus was the most prevalent (25.0%) isolated bacteria from pus followed by E coli, Pseudomonas, Acinatobactor species and Klebsiella species contributing to 16.5%, 14.6%, 4.7% and 0.9% isolates respectively[2]. Similarly, Mantravadi H B *et al*[12] revealed similar results of S. aureus as commonly occuring pathogen (37.2%) similar to studies by Rao DVMVSVR *et al*[13], Tiwari P *et al*[27], Lee CY *et al*[28] and Mahmood A[29]. However, Agnihotri N *et al* found Pseudomonas species to be more prevalent than S aureus [30] Another study conducted by Basu S *et al*[31] showed that Pseudomonas and E coli spp to be the most prevalent pathogens in wound infections which is in contradiction to the present study results. In a study conducted in Kathmandu Raza MS *et al* found *E coli* to be the most commonly occurring pathogen[15].

Gram Negative dominance :

In our study half isolates belonged to gram Negative bacteria (GNB). Overall, similar results have been reported by Khanam RA *et al*[2]. Mohammed A *et al* observed more than half (57%) of the isolates as GNB[18]. According to Sudhaharan S *et al* GNBs were isolated in 68.3%, *E coli* being the major one (38.6%); gram positive bacteria (GPB) were isolated in 31.6% of cases and S aureus was commonly occuring organism (91.7%) out of which 43.34% was MRSA[20]. Subha M *et al*,[25] Ghosh A *et al*,[26] Basu S *et al*[31] and Zubair M *et al*[32] also reported Pseudomonas and *E coli* spp. to be the widely prevalent pathogen in wound infections.

Sensitivity pattern :

In present study the most predominant isolate S. aureus showed high sensitivity to Erythromycin, Vancomycin, Doxycycline. The gram negative species dominated by Klebsiella sp (23.91%), Acinetobactor sp (13.04%), P aeroginosa (6.52%) and *E coli* (4.35%) were found to have maximum sensitivity to Colistin, Imipenem, Amikacin, Levoûoxacin and Gentamicin.

Khanam RA *et al* observed S aureus to have high resistance to penicillin (up to 84.5%), moderate sensitivity (58.3%) to Erythromycin while fair sensitivity to Vancomycins like clindamycin. Highest level of sensitivity was revealed towards high- end drugs such as Linezolid and Vancomycin.

While Streptococcus is sensitive to most of the drugs[2]. Rao DVMVSVR *et al* also found S. aureus highly resistant to Penicillin (84.62%), Erythromycin (84.62%), and sensitive to Clindamycin (65.38%) and Vancomycin (100%)[13]. The antibiogram in another study revealed that the S aureus was mostly susceptible to Vancomycin (89%) followed by Gentamicin (86%), Cefoxitin (82%), and resistant to Penicillin. The antibiogram of Pseudomonas revealed that it was more sensitive to Imipenem (97%) and resistant to Cotrimoxazole. Enterobacteriaceae were sensitive to Imipenem[17]. A study conducted at Peswar, Pakistan explored that Gram-positive isolates were resistant to Ampicillin (86.4%), Amoxicillin (83%), Penicillin (81.3%), Oxacillin (74.6%), and Tetracycline (59.4%), but Gram-negative isolates resistant to Amoxicillin (97.4%), Ampicillin (94.8%), Tetracycline (72.7%), Trimethoprim/sulfamethoxazole (66%), and Chloramphenicol (54.5%) were also noted[18].

In another study, 66.2% isolates were resistant to Tetracycline, followed by 59.8% for Ampicillin, 59.1% for Cotrimoxazole, 51.7% for Penicillin; least resistant being 6.3% for Gentamicin[19]. From Peswar study Khaan I et al. revealed that majority of isolates were observed to be resistant to three or more classes of antibiotics. S. aureus were resistant to Amoxicillin (82%), Ofloxacin (80%), Sparfloxacin (78%), Ciprofloxacin (71%), Levofloxacin (46%) and Gentamicin (36%). Sensitivity to Tygacil and Linezolid was universal, and isolates showed low resistance to sulzone (2%), Oxacillin (3%), Vancomycin (4%), Fusidic acid (5%), Clarithromycin (7%), Erythromycin (8%), Cefoxitin (9%), Amikacin (15%), Cefaclor (15%) and Cephradine (19%)[21].

According to Kumari Pilli H P *et al* S aureus showed

maximum sensitivity to antibiotics like Linezolid (83.3%) and Teicoplanin (50%)[23].

Antibiotic sensitivity pattern as explored by Shama M *et al* showed that Cefaperazone/ Sulbactum was highly effective drug against commonest gram negative isolates *E coli* (57.5%), followed by Proteus sp (31.5%) - Penicillin and Ampicillin were highly effective drugs[24]. Subha M *et al* showed MRSA was 17.5% and 100% sensitive to Vancomycin. Around one fifth (23.61%) of E coli and 25% of K pneumoniae ESBL producers. Imipenem and Meropenem were effective for majority of the gram negative isolates[25].

Study in Nigeria carried out by Taiwo S S *et al* revealed 99.6% resistance to Ampicillin and 33.1% to Oxacillin, 72.7% to Erythromycin but 100% sensitivity to Vancomycin and more than 98% to Linezolid. GNBs were highly resistant to b-lactams whereas Carbapenems are still reactive, however increasing resistance was observed to Meropenem[33].

Amongst the aminoglycosides Amikacin showed good sensitivity in spite of rising resistance to Gentamicin and Tobramycin. Drug combination such as Piperacillin plus Tazobactam and Cefoperazone plus Sulbactum was found to be good[2]. In their studies Taiwo SS *et al*[33], Rao DVMVSVR *et al*[13], and Basu S *et al*[31] also corroborated these findings.

Razza MS *et al* showed that all isolates of S aureus was sensitive to Vancomycin and Aminoglycosides. About two-fifth (41.66%) S aureus isolates was MRSA High resistance against Cephalexin (75% - 100%) and Ceftriaxone (25% - 100%) was detected among all gram negative isolates. Grossly 66.7% were multi-drug resistant isolates[15].

Limitations of the study : The study was conducted in one of the Medical Colleges of Kolkata which caters a small segment of the total patients turn out for treatment in all other health facilities in the capital city of West Bengal. So, the only constraint was in its external validity. Other factors of antibiotic resistance like duration and compliance to treatment, comorbidity, nutritional status of the patients etc. couldn't be taken into consideration in this small study. A large scale multicentre study encompassing all these correlates of antibiotic usage may be tried for drawing a reliable and valid inference.

Conclusion

Pyogenic infections are frequently encountered in day to day clinical challenges and most of them are resistant to one or more antibiotics, thus limiting treatment options. The finding of the present study is helpful to guide for developing antibiotic policy and empirical therapy and thus reducing morbidity of patients. A correct antibiotic strategy and the avoidance of inappropriate antimicrobial usage are mandatory to mitigate the containment of antibiotic resistance in the community, also keeping newer antibiotics in reserve for use only against strains that are resistance to the common antibiotics.

Conflict of Interest : None

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