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Profile of Cutaneous Bacterial Flora in Pemphigus Patients

Srujana Mohanty[1] Swarnatrisha Saha[1] Shehnaz Firdaus[1] Chandra Sekhar Sirka[2]

[1] Department of Microbiology, All India Institute of Medical Sciences Bhubaneswar, Bhubaneswar, Odisha, India
[2] Department of Dermatology, All India Institute of Medical Sciences Bhubaneswar, Bhubaneswar, Odisha, India

Address for correspondence Srujana Mohanty, MD, Department of Microbiology, All India Institute of Medical Sciences Bhubaneswar, Bhubaneswar 751019, Odisha, India (e-mail: srujana_micro@yahoo.co.in).

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Abstract

Objectives Pemphigus, a group of autoimmune bullous diseases, can be fatal, resulting from overwhelming opportunistic infection of lesions secondary to cutaneous bacterial infections. This study aimed to look into the cutaneous bacterial infection profile of pemphigus patients as timely identification and appropriate treatment can play a major role in reducing mortality.

Materials and Methods Pus samples/swabs received from patients with pemphigus over a 2-year period from July 2018 to June 2020 were subjected to standard microbiological culture techniques and susceptibility testing. The frequency of isolation and susceptibility profile of the different bacterial pathogens toward various antimicrobial agents were interpreted and analyzed as per the Clinical and Laboratory Standards Institute's guidelines.

Results Samples from 315 patients were received during the study period comprising of 203 (64.4%) males and 112 (35.5%) females. Of 211 samples which were culture-positive, a total of 245 bacterial isolates were obtained, comprising of 158 Gram-positive cocci and 87 Gram-negative bacilli. *Staphylococcus aureus* (138, 56.3%) was the most common isolate followed by *Pseudomonas aeruginosa* (41, 16.7%) and *Escherichia coli* (16, 6.5%). Methicillin resistance was observed in 24.6% *Staphylococcus aureus* isolates and carbapenem resistance in 9.5 to 14.6% Gram-negative bacilli.

Conclusions Study findings emphasize the need for continuous monitoring of cutaneous pemphigus lesions for appropriate choice of antimicrobial therapy.

Keywords
- methicillin-resistant *S. aureus*
- MRSA
- pemphigus
- *Staphylococcus aureus*
- susceptibility

Introduction

Pemphigus (derived from the Greek word "*pemphix*" meaning bubble) is a heterogeneous group of bullous diseases, autoimmune in nature, which affect the skin and mucous membranes.[1] The condition is characterized by acantholysis (cell adhesion loss) and development of blisters within the epidermis; and is mediated by immunoglobulin G autoantibodies formed against adhesion molecules.[1,2] Incidence has been estimated to vary from 0.09 to 1.8% among the dermatology outpatient attendees and 0.76 to 16.1 cases per million in the general population.[3,4] Systemic glucocorticoids and immunosuppressants are the mainstay of therapy of this chronic disorder, the advent of which has largely led to the improvement of the overall prognosis of patients suffering from pemphigus. However, long-term use of

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immunosuppressants in high doses and the resultant immunocompromised state of patients predispose to high risk of infections which account for 34.3 to 55.5% of all deaths.[4] Moreover, infectious complications are also liable to occur due to the disease process itself because of disruption of the epidermal barrier. Thus, if not treated, pemphigus can be fatal, usually from overwhelming opportunistic infection such as septicemia and pneumonia, secondary to cutaneous bacterial infections.[4,5] Therefore, timely identification of infection, its causative bacteriological agent, and appropriate treatment can play a major role in reducing the mortality. In this study, we aim to look into the cutaneous bacterial infection profile in pemphigus patients presenting to a tertiary-level hospital and examine their respective pattern of susceptibility to various antimicrobial agents.

Materials and Methods

The retrospective study, exempted from review by the Institutional Ethics Committee, was performed from July 2018 to June 2020 over a 2-year time frame in the department of microbiology of a tertiary-level health care setting in the Eastern region of India. Pus samples or swabs received from patients with pemphigus after swabbing the lesions with sterile normal saline were subjected to culture using standard microbiological techniques.[6] Identification and antimicrobial susceptibility testing of the bacterial isolates was performed per manufacturer's instructions by VITEK-2 Compact automated system (bioMérieux, Marcy l'Etoile, France). The resultant minimum inhibitory concentrations were interpreted as per the Clinical and Laboratory Standards Institute's (CLSI) breakpoints as susceptible, intermediate, or resistant.[7] Tigecycline results were interpreted as per the European Committee on Antimicrobial Susceptibility Testing guidelines.[8] Susceptibility testing for antimicrobial agents for which CLSI breakpoints were available, but not included in the VITEK panel, were supplemented by the use of disc-diffusion or EzyMIC testing (HiMedia, Mumbai, Maharashtra, India). Strains used for quality control purposes were *Staphylococcus aureus* ATCC 29213, *S. aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. Results of strains having intermediate resistance were included in the percentage of resistant isolates.

Results and Discussion

During the period of study, samples from 315 patients were received, which were comprised of 203 (64.4%) males and 112 (35.5%) females. The lowest age was that of a 1-year-old male child and the highest a 87-year-old female patient (age range, 1–87 years). One hundred six (33.6%) patients were admitted, while 209 (66.3%) were from the outpatient department. A total of 252 (80.0%) samples were culture-positive, with growth of diphtheroid, other commensal skin flora, environmental bacillus species, and more than three morphotypes in 41 samples which were not processed further. The remaining 211 samples yielded 245 bacterial isolates comprising of 158 Gram-positive cocci and 87 Gram-negative bacilli. Culture from 34 (13.5%) patients grew two organisms each, while that from the remaining 177 (70.2%) grew a single organism. *S. aureus* (138, 56.3%) was found to be isolated most frequently, followed by *P. aeruginosa* (41, 16.7%), *E. coli* (16, 6.5%), and *Klebsiella pneumoniae* (14, 5.7%) (►**Table 1**). Apart from *S. aureus*, other Gram-positive organisms included *Streptococcus pyogenes* (9, 3.7%), *Enterococcus* spp. (7, 2.9%), and coagulase-negative staphylococci (4, 1.6%). Total number of *Enterobacterales* isolated was 42 (►**Table 1**).

Analysis of susceptibility profile among the predominant Gram-positive cocci revealed that methicillin resistance occurred in 24.6% (34/138) of *S. aureus* isolates (►**Table 2**). The resistance pattern of *S. aureus* to other antibiotics was as follows: penicillin (116, 84.1%), erythromycin (97, 70.3%), clindamycin (76, 55.1%), ciprofloxacin (84, 60.9%), levofloxacin (69, 50.0%), trimethoprim-sulfamethoxazole (30, 21.7%), chloramphenicol (29, 21.0%), gentamicin (14, 10.1%), and tetracycline (3, 2.2%). *S. pyogenes* were the next most common Gram-positive bacteria from the pemphigus lesions and all ($n = 9$, 100%) were susceptible to penicillin, ceftriaxone, vancomycin, linezolid, and tigecycline. Resistance was observed to erythromycin, clindamycin, chloramphenicol, levofloxacin, and tetracycline in 4 (44.4%), 3 (33.3%), 2 (22.2%), 1 (11.1%), and 1 (11.1%) isolates of *S. pyogenes*. The D-test (indicating inducible clindamycin resistance) was positive in 43 (31.1%) *S. aureus* and 1 (11.1%) *S. pyogenes* isolates, respectively. As regards enterococci, only one (14.3%) exhibited simultaneous penicillin and high-level gentamicin resistance; rest being susceptible to all the tested antimicrobials. As regards Gram-negative bacilli, among the 42 *Enterobacterales* isolates, 23 (54.8%), 6 (14.3%), and 4

Table 1 Distribution of bacterial isolates in cutaneous infections of 315 pemphigus patients

Species	Number of isolates (%)
Staphylococcus aureus	138 (56.3)
Pseudomonas aeruginosa	41 (16.7)
Escherichia coli	16 (6.5)
Klebsiella pneumoniae	14 (5.7)
Streptococcus pyogenes	9 (3.7)
Enterococcus species[a]	7 (2.9)
Proteus species[b]	5 (2.0)
Coagulase-negative staphylococci[c]	4 (1.6)
Citrobacter species[d]	4 (1.6)
Enterobacter cloacae	3 (1.2)
Acinetobacter baumannii complex	3 (1.2)
Burkholderia cepacia	1 (0.4)
Total	245

[a]*E. faecalis*, 5; *E. faecium*, 2.
[b]*P. mirabilis*, 4; *P. vulgaris*,1.
[c]*S. epidermidis*, 2; *S. hominis*, 2.
[d]*C. koseri*, 3; *C. freundii*, 1.

Table 2 Antimicrobial resistance of bacterial isolates from pemphigus lesions

Gram-positive organisms (% resistant)			
Antimicrobial agent	***Staphylococcus aureus* (*n* = 138)**	***Streptococcus pyogenes* (*n* = 9)**	***Enterococcus species* (*n* = 7)**
Penicillin	116 (84.1)	0	1 (14.3)
Oxacillin	34 (24.6)	–	–
Ceftriaxone	–	0	–
Gentamicin	14 (10.1)	–	–
Gentamicin high-level (synergy)	–	–	1 (14.3)
Ciprofloxacin	84 (60.9)	–	0
Levofloxacin	69 (50.0)	1 (11.1)	0
Erythromycin	97 (70.3)	4 (44.4)	0
Clindamycin	76 (55.1)	3 (33.3)	–
Tetracycline	3 (2.2)	1 (11.1)	0
Rifampicin	4 (2.9)	–	–
Trimethoprim-sulfamethoxazole	30 (21.7)	1 (11.1)	
Chloramphenicol	29 (21.0)	2 (22.2)	–
Vancomycin	0	0	0
Teicoplanin	0	–	0
Linezolid	0	0	0
Tigecycline	0	0	0
Daptomycin	0	0	0
D-zone test positive	43 (31.1)	1 (11.1)	–
Gram-negative organisms (% resistant)			
Antimicrobial agent	***Enterobacterales* (*n* = 42)**	***Pseudomonas aeruginosa* (*n* = 41)**	***Acinetobacter baumannii* (*n* = 3)**
Cefuroxime	32 (76.2)	–	–
Ceftriaxone	23 (54.8)	–	–
Cefepime	23 (54.8)	10 (24.4)	0
Ceftazidime	–	10 (24.4)	1 (33.3)
Aztreonam	–	8 (19.5)	1 (33.3)
Piperacillin/tazobactam	6 (14.3)	6 (14.6)	2 (66.6)
Ticarcillin/clavulanate	–	6 (14.6)	–
Amikacin	2 (4.8)	3 (7.3)	0
Gentamicin	4 (9.5)	4 (9.8)	0
Ciprofloxacin	21 (50)	8 (19.5)	0
Trimethoprim-sulfamethoxazole	13 (30.9)	–	0
Imipenem	4 (9.5)	6 (14.6)	0
Meropenem	4 (9.5)	6 (14.6)	0
Doripenem	4 (9.5)	6 (14.6)	0
Colistin	0[a]	0	0
Tigecycline	0[a]	–	–

[a]Not tested in *Proteus* species.

(9.5%) were resistant to the third-generation cephalosporins, piperacillin-tazobactam, and carbapenems, respectively (►**Table 2**). Resistance to ciprofloxacin, trimethoprim-sulfamethoxazole, gentamicin, and amikacin was observed in 21 (50.0%), 13 (30.9%), 4 (9.5%), and 2 (4.8%) isolates, while none were resistant to colistin and tigecycline (except *Proteus* which is intrinsically resistant to both). Isolates of *P. aeruginosa* ($n = 41$) exhibited the following resistance pattern:

ceftazidime (10, 24.4%), cefepime (10, 24.4%), ciprofloxacin (8, 19.5%), aztreonam (8, 19.5%), piperacillin-tazobactam (6, 14.6%), carbapenem (6, 14.6%), gentamicin (4, 9.8%), and amikacin (3, 7.3%) (►**Table 2**). The lone isolate of *Burkholderia cepacia* complex was susceptible to its tested panel of antibiotics, namely, ceftazidime, meropenem, ticarcillin-clavulanate, levofloxacin, and chloramphenicol.

The most common comorbidity in pemphigus patients is infection, which may lead to increased morbidity and mortality.[9] In the current study, 211 of 315 (66.9%) patients had clinically and microbiologically proven infected pemphigus lesions substantiating the large burden of cutaneous infection in these patients. Furthermore, 33.6% of patients were hospitalized, which emphasizes the severity of the infection leading to hospital admission. Infections have been observed in 60.6 to 68% of pemphigus patients in various studies from the world, with cutaneous infections observed in 10.32 to 71.4% of them.[5,10–12]

In the current study, *S. aureus* (56.3%) was the most common organism isolated from pemphigus patients followed by *P. aeruginosa* (16.7%) which is consistent with many other studies.[5,11–15]

In the study by Esmaili et al,[11] Qadim et al,[5] and Solanki et al,[13] *S. aureus* accounted for 93.7, 82.9, and 72% of the isolates, which is quite high than that observed in the current study. However, in the study by Kiran et al,[12] *S. aureus* accounted for a lower frequency of 40.8% of the isolates.[12] This difference in the frequency isolation may be due to local regional variation in the bacterial population related to specific patient groups or the prevalent pattern of antimicrobial prescription practices or therapy leading to selection of isolates.

Frequency of methicillin resistance (24.6%) observed in the present study, is similar to a very recent study on skin and soft tissue infections from North India reporting 24.18% methicillin resistance among *S. aureus* isolates.[16] However, an evaluation of *S. aureus* infections in pemphigus patients by Motallebi et al revealed a higher methicillin resistance of 43.2%.[17] In the current study, maximum resistance in *S. aureus* isolates was observed for penicillin (84.1%) followed by erythromycin (70.3%) and ciprofloxacin (60.9%). A study, by Kiran et al, also found similar resistance pattern of penicillin (90%), erythromycin (55%), and ciprofloxacin (55%) in pemphigus patients.[12] Li et al found resistance rates of penicillin G, erythromycin, and clindamycin to be 91.9, 75.8, and 45.2%, respectively, among 62 *S. aureus* isolated from hospitalized patients with pemphigus.[4] *S. pyogenes*, the second most common Gram-positive bacteria found in the current study displayed maximum resistance to erythromycin and clindamycin (44.4 and 33.3%, respectively), which is a persisting problem as observed by other researchers.[18,19] Susceptibility to penicillin, has fortunately been retained in these studies[18,19] as well as in our study which is the antimicrobial agent of choice.

Among Gram-negative bacteria, a high resistance was observed for third-generation cephalosporins (24.4–54.8%), ciprofloxacin (19.5–50.0%), and trimethoprim-sulfamethoxazole (30.9%) as that observed by Esmaili et al.[11] In contrast, 100% of the *P. aeruginosa* isolates were sensitive to ceftazidime and ciprofloxacin as well as to amikacin and gentamicin in the study by Kiran et al.[12] Colistin and tigecycline resistance was not exhibited by any of the isolates in the current study (except *Proteus* species which are intrinsically resistant to both). Carbapenem resistance was low (9.5–14.6%), which was also found in the study by Kiran et al.[12] However, at other places, the burden of carbapenem-resistant Gram-negative bacteria in various types of skin and soft tissue infections is increasing.[20] Carbapenem-resistant *Enterobacteriaceae* with distinct carbapenemase-encoding genes exhibit variation in their geographic spread, as well as display differing susceptibility to the newer therapeutic agents such as the newer beta-lactam combination agents and cefiderocol.[20–23] Hence, there is a need for molecular characterization of carbapenemase genes in carbapenem-resistant bacteria in routine clinical practice. It is known that infection by multidrug-resistant organisms like methicillin-resistant *S. aureus* and carbapenem-resistant organisms may be associated with serious systemic complications and multiorgan failure leading to prolonged hospitalization, higher economic burden, and increased mortality.[20–22] So outlining the profile of antimicrobial susceptibility of the isolates and administration of appropriate antibiotics are recommended for better patient outcome.

There are certain limitations of the study in view of its retrospective design. It would have been better if any relationship could have been delineated between the number and type of isolates and the duration of steroid treatment in the patients. Molecular characterization of the resistant isolates or their resistance mechanisms would also have contributed to crucial information. To conclude, cutaneous pemphigus lesions are infected by a large variety of bacterial species that demonstrate varying antimicrobial susceptibility pattern, emphasizing the need for continuous monitoring to help in the appropriate choice of empiric or definitive therapy.

Ethics Approval

The study obtained review exemption by the Institutional Ethics Committee (IEC) of our Institute, i.e., the Institutional Ethics Committee of All India Institute of Medical Sciences, Bhubaneswar, Odisha, India. Reference number - T/IM-NF/Micro/20/123 dated 24.09.2020.

Authors' Contributions

- S.M. - provided substantial contribution to the concept and design of the study, acquisition, analysis, and interpretation of data for the work, did the literature search, and revised the work for important intellectual content. She is the corresponding author who gave the final approval for the manuscript to be published.
- S.S. - provided substantial contribution to the acquisition, analysis, and interpretation of data for the work, did the literature search, and drafted the initial manuscript.
- S.F. - provided substantial contribution to the acquisition, analysis, and interpretation of data for the work,

did the literature search, and revised the work for important intellectual content.

- C.S.S. – was the treating physician and contributed to the acquisition, analysis, and interpretation of data for the study as well as critically revised the work for important intellectual content.

Note

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Conflict of Interest

None declared.

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References

1 Korman N. Pemphigus. J Am Acad Dermatol 1988;18:1219–1238
2 Didona D, Maglie R, Eming R, Hertl M. Pemphigus: current and future therapeutic strategies. Front Immunol 2019;10:1418
3 Kanwar AJ, De D. Pemphigus in India. Indian J Dermatol Venereol Leprol 2011;77(04):439–449
4 Li F, Wu Y, Bian W, et al. Features and associated factors of bacterial skin infections in hospitalized patients with pemphigus: a single-center retrospective study. Ann Clin Microbiol Antimicrob 2020;19(01):46
5 Qadim HH, Hasani A, Zinus BM, Orang NJ, Hasani A. Etiology of pyrexia in pemphigus patients: a dermatologist's enigma. Indian J Dermatol Venereol Leprol 2012;78(06):774
6 Collee JG, Miles RS, Watt B. Tests for the identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, eds. Mackie and McCartney Practical Medical Microbiology. 14th ed. London: Churchill Livingstone; 1996:131–145
7 CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI Supplement M100 Wayne, PA: Clinical and Laboratory Standards Institute; 2020
8 European Committee on Antimicrobial Susceptibility Testing. Breakpoint Tables for Interpretation of MICs and Zone Diameters, 2020, Version 10.0. Accessed April 12, 2023, at: http://www.eucast.org
9 Ren Z, Narla S, Hsu DY, Silverberg JI. Association of serious infections with pemphigus and pemphigoid: analysis of the Nationwide Inpatient Sample. J Eur Acad Dermatol Venereol 2018;32(10):1768–1776
10 Belgnaoui FZ, Senouci K, Chraibi H, et al. Predisposition to infection in patients with pemphigus. Retrospective study of 141 cases [in French]. Presse Med 2007;36(11 Pt 1):1563–1569
11 Esmaili N, Mortazavi H, Noormohammadpour P, et al. Pemphigus vulgaris and infections: a retrospective study on 155 patients. Autoimmune Dis 2013;2013:834295
12 Kiran KC, Madhukara J, Abraham A, Muralidharan S. Cutaneous bacteriological profile in patients with pemphigus. Indian J Dermatol 2018;63(04):301–304
13 Solanki RB, Shah YB, Shah AN, Jain V. Bacterial culture and sensitivity in pemphigus. Indian J Dermatol Venereol Leprol 1997;63(02):89–90
14 Ahmed AR, Moy R. Death in pemphigus. J Am Acad Dermatol 1982;7(02):221–228
15 Asati DP, Sharma VK, Khandpur S, Khilnani GC, Kapil A. Clinical and bacteriological profile and outcome of sepsis in dermatology ward in tertiary care center in New Delhi. Indian J Dermatol Venereol Leprol 2011;77(02):141–147
16 Agrawal SK, Panigrahy A, Perumalla S, Kapil A, Dhawan B. Microbiological profile and antibiotic resistance pattern of skin and soft-tissue infections: A study from Northern India. J Lab Physicians 2018;10(04):471–472
17 Motallebi M, Alibolandi Z, Aghmiyuni ZF, van Leeuwen WB, Sharif MR, Moniri R. Molecular analysis and the toxin, MSCRAMM, and biofilm genes of methicillin-resistant Staphylococcus aureus strains isolated from pemphigus wounds: a study based on SCCmec and dru typing. Infect Genet Evol 2021;87:104644
18 Silva-Costa C, Friães A, Ramirez M, Melo-Cristino J. Macrolide-resistant Streptococcus pyogenes: prevalence and treatment strategies. Expert Rev Anti Infect Ther 2015;13(05):615–628
19 Tsai WC, Shen CF, Lin YL, et al. Emergence of macrolide-resistant Streptococcus pyogenes emm12 in southern Taiwan from 2000 to 2019. J Microbiol Immunol Infect 2021;54(06):1086–1093
20 Del Giacomo P, Losito AR, Tumbarello M. The role of carbapenem-resistant pathogens in cSSTI and how to manage them. Curr Opin Infect Dis 2019;32(02):113–122
21 Veeraraghavan B, Pragasam AK, Bakthavatchalam YD, et al. Newer β-Lactam/β-Lactamase inhibitor for multidrug-resistant gram-negative infections: challenges, implications and surveillance strategy for India. Indian J Med Microbiol 2018;36(03):334–343
22 Bakthavatchalam YD, Routray A, Mane A, et al. In vitro activity of Ceftazidime-Avibactam and its comparators against Carbapenem resistant Enterobacterales collected across India: results from ATLAS surveillance 2018 to 2019. Diagn Microbiol Infect Dis 2022;103(01):115652
23 Fröhlich C, Sørum V, Tokuriki N, Johnsen PJ, Samuelsen Ø Evolution of β-lactamase-mediated cefiderocol resistance. J Antimicrob Chemother 2022;77(09):2429–2436

