incubated at 37°C overnight for the growth of pathogenic bacteria, which were identified according to the standard method used for bacteria and concomitantly for fungi (Figs. 1 and 2). Antibiotic susceptibility tests of isolated bacteria were done according to Clinical Laboratory Standard Institute guidelines, as described by Mishra et al. and Rath et al. [12,13]. Standard antimicrobial discs (HiMedia, Mumbai) used for *S. aureus* were amikacin, amoxyclav, chloramphenicol, ciprofloxacin, cotrimoxazole, gentamicin, levofloxacin, linezolid, oxacillin, and vancomycin. Antimicrobial discs used for P. aeruginosa were amikacin, amoxyclav, ceftriaxone, ciprofloxacin, ceftazidime, gentamicin, piperacillin, netilmicin, ofloxacin, and tobramycin.

Antibiotic sensitivity and detection of MRSA

The standard MTCC number 7443 strain and all the isolated *S. aureus* strains were subjected to antibiotic sensitivity tests with antibiotics, by the Kirby-Bauer method (disc diffusion) detailed previously.

Identification of fungi

Direct microscopic examination of cotton swabs with samples was carried out by mounting sample lots treated with 1–2 drops of 10–20% KOH for 15–30 min. Each specimen was inoculated on two sets of Sabouraud dextrose agar slopes, one set with chloramphenicol, and the other set with cycloheximide (chloramphenicol - 0.05 mg/ mL and cycloheximide - 0.5 mg/mL). Cultures were incubated at room temperature for 4–6 weeks and were observed regularly for possible growth. Fungal isolates were identified on the basis of duration of growth and surface morphology of colonies, as well as pigment production on the reverse and microscopic examination of hyphae in lacto phenol cotton blue preparation [13].

RESULTS

From 621 collected samples, 509 bacterial and fungal colonies grew on agar plates, and no microbial growth was seen with 112 samples. There were 468 bacterial and 41 fungal isolates in total. The most common causal bacteria isolated were 250 isolates of *S. aureus* with and 74 isolates of *P. aeruginosa*; and 98 isolates of *S. aureus* were MRSA. Of 509 samples, isolated bacteria were in decreasing order (with number of isolated strains): *Staphylococcus aureus* (250) > *P. aeruginosa* (74) > *Acinetobacter baumannii* (48) > *Escherichia coli* (24) > *Klebsiella pneumoniae* (20)> *Enterobacter aerogenes* (18) > *Proteus vulgaris* (15) > *Citrobacter* sp. (10) > *Enterococcus faecalis* (09). Fungi accounted for 13 isolates of *A. niger* and 28 isolates of *C. albicans* from 509 growth-yielding samples (Table1).

Antibiograms of the most common bacteria, *P. aeruginosa* and *S. aureus* (other than MRSA) were presented. The susceptibility rate of *P. aeruginosa* to tobramycin 10 µg/disk had 91%, followed by ciprofloxacin 5 µg/disk 79% and piperacillin 100 µg/disk 77% and 100% *S. aureus* isolates were susceptible to vancomycin 30 µg/disk, followed by 88% to levofloxacin 5 µg/disk and 77% isolates to amoxyclav 30 µg/disk. Thus, all isolated strains of MRSA were multidrug resistant (MDR) (Fig. 3). With a cohort of 98 MRSA strains, the minimum inhibitory concentration (MIC) range against oxacillin was 16–512 µg/mL, the MIC range of methicillin-sensitive *S. aureus* was 1–4 µg/mL. These MIC values confirmed the presence of MRSA strains, as the breakpoint for being resistant to oxacillin was ≥4 µg/mL (Table 2 and 3).

The antifungal susceptibility rate of *A. niger* to amphotericin B (AMB) was 82%, followed by liposomal AMB 75% and itraconazole (ITC) 63%, voriconazole (VRC) 55%, posaconazole (POS) 48%, and caspofungin (CPF) 32%; similarly, susceptibility rate of *C. albicans* to AMB was 86%, followed by liposomal AMB 77% and ITC 69%, VRC 62%, POS 57%, and CPF 49% resistance (Fig. 4, Table 4).

DISCUSSION

MDR strains of MRSA and *P. aeruginosa* had emerged nosocomially, as post-operative infection in orthopedic surgery patients. Obviously, the nosocomial emergence of MDR strains of bacteria is basically associated with substantial morbidity, increased the length of hospital stay and

Table 1: Growth of bacteria in cultures of wound swabs of patients admitted to orthopedic wards

Organisms	MTCC strain number	Total isolates n=509 (100)
Enterococcus sp.	439	09 (01.76)
MRSA		98 (19.25)
MSSA	7443	152 (29.86)
A. baumannii	1425	48 (09.43)
Citrobacter sp.	1658	10 (01.96)
E. aerogenes	2990	18 (03.53)
E. coli	443	24 (04.71)
Klebsiella sp.	2275	20 (03.92)
P. vulgaris	1771	15 (02.94)
P. aeruginosa	1688	74 (14.53)
A. niger	872	13 (02.55)
C. albicans	1425	28 (05.50)

MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-sensitive *Staphylococcus aureus*, the standard strain, percent values are in parenthesis, n or total isolates=509, from the total 621 samples; the rest 112 samples had no growth. *P. aeruginosa: Pseudomonas aeruginosa, A. niger: Aspergillus niger, E. coli: Escherichia coli, E. aerogenes: Enterobacter aerogenes, P. vulgaris: Proteus vulgaris, A. baumannii: Acinetobacter baumannii, C. albicans: Candida albicans*

Table 2: Antibiogram of resistance *S. aureus* and *P. aeruginosa*

Antibiotics	*S. aureus*	*P. aeruginosa*
Ac	39	26
Ak	23	28
Cf	Nd	24
Ch	29	Nd
Cot	34	Nd
Cp	38	21
Cz	Nd	32
Ge	25	36
Le	12	Nd
Lz	32	Nd
Ne	Nd	25
Of	Nd	35
Ox	42	Nd
Pi	Nd	23
Tb	Nd	09
V	0	Nd

Antibiotic in µg/disc: Ac: Amikacin 30, Ak: Amoxyclav 30, Cf: Ceftriaxone 30, Ch: Chloramphenicol 30, Cp: Ciprofloxacin 5, Cot: Cotrimoxazole 25, Cz: Ceftazidime 30, Ge: Gentamicin 10, Le: Levofloxacin 5, Lz: Linezolid 30, Ne: Netilmicin 30, Of: Ofloxacin 5, Ox: Oxacillin 1, Pi: Piperacillin 100, Tb: Tobramycin 10, V: Vancomycin 30, Nd: Not done. *P. aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus*

Table 3: Detection of MRSA and MSSA isolates based on MIC values from the presence of oxacillin in 12×8 µl plates

Well	Oxacillin (µg/mL)	Number of isolates	
		MRSA=98	MSSA=152
1	0	98	152
2	≤0.25	–	–
3	0.5	–	–
4	1	–	68
5	2	–	36
6	4	–	48
7	8	–	–
8	16	12	–
9	32	18	–
10	64	20	–
11	128	22	–
12	≥ 256	26	–

The oxacillin stock solution, 512 µg/mL was serially diluted at each successive well, from the 12th well for a final concentration of 0.25 µg/mL oxacillin at the 2nd well was obtained; –, no growth. MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-sensitive *Staphylococcus aureus*, MIC: Minimum inhibitory concentration

Table 4: Antifungal agents used against *A. niger* and *C. albicans*

Antifungal	*A. niger*	*C. albicans*
AMB	82	86
1- AMB	75	77
ITC	63	69
VRC	55	62
POS	48	57
CPF	32	49

Antifungal agents: AMB: Amphotericin B, l- AMB: Liposomal AMB, ITC: Itraconazole, VRC: Voriconazole, POS: Posaconazole, CPF: Caspofungin. *A. niger: Aspergillus niger, C. albicans: Candida albicans*

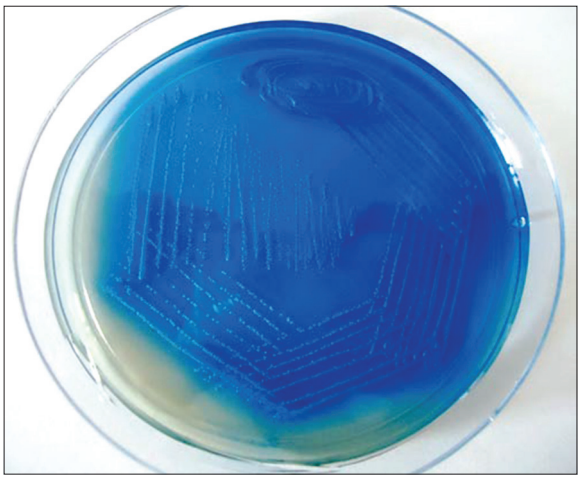

Fig. 1: Methicillin-resistant *Staphylococcus aureus* on MeReSa chromogenic agar

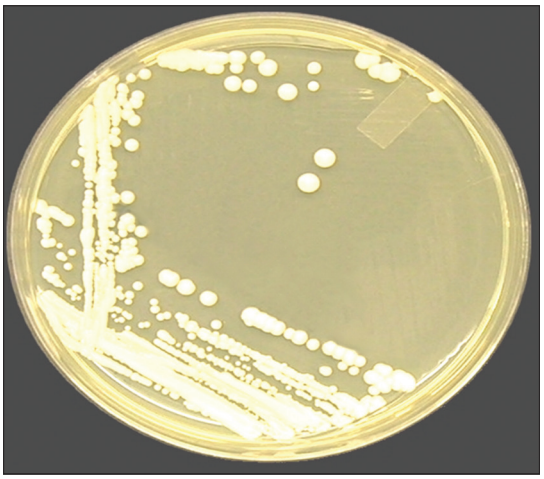

Fig. 2: *Candida albicans* in potato dextrose agar media

a higher incidence of amputation and graft removal, particularly in orthopedic surgery patients. Thus, greater emphasis on pre-operative screening protocols for colonization of these pathogens should be considered, accordingly for infection control measures aggressively, with minor alteration of pre-operative prophylactic antimicrobial uses; and meticulous post-operative surveillance for MRSA infection is a dire necessity for this superbug of health domain. Antimicrobial treatment should include empiric coverage for MRSA in institutions where MRSA is endemic. It was found that in a study from Serbia, S. *aureus* was recorded as the most frequently isolated pathogen from SSIs isolated pathogens of which 43.7% were MRSA; 81.5% *P. aeruginosa* strains were resistant to fluoroquinolones and carbapenems [14]. A prolonged pre-operative hospital stay with exposure to a hospital environment had been shown to increase the risk from SSI wound contamination [15]. It was also reported a higher rate of SSI in patients with a prolonged pre-operative hospital stay. Indeed, prolonged pre-operative hospital stay leads to colonization with antimicrobial resistant microorganisms by providing increased opportunity for ultimate bacterial colonization [16]. Eventually, this may to lead septicemia/bacteremia that may to lead to amputation in the absence of emulating control required for MDR bacteria.

In the present study, *S. aureus* was predominant in surgical sites, followed by *P. aeruginosa* and *Klebsiella* sp., while, *E. coli*, *Citrobacter,* and *Proteus* sp. were too isolated from surgical sites, corroborating another report [17]. Many studies have reported *S. aureus* as the most common isolate from the post-operative wound infection [18]. Furthermore, the incidence of isolated Gram-negative bacteria in surgical wounds can be attributed to be acquired from patient's normal endogenous microflora [18].

In 5 years study from Saudi Arabia, of total 830 patients, 29.11% MRSA, 21.5% *Acinetobacter* sp., 18.9% *Pseudomonas* sp., and 17.7% *Enterococcus* sp. were recorded. Emergency surgical procedures carried the greatest risk with *Staphylococcus* sp. and *Acinetobacter* sp. being the most common infecting bacteria from treatments of dirty wounds. Similar to MRSA, methicillin-resistant *Staphylococcus epidermidis* strains were reportedly frequently nosocomially in orthopedic wards [19,20]. Resistant Gram-negative forms of bacteria were increasingly prevalent in hospitals and communities [20]. As known, tibial plateau fractures are challenging of treatment due to the high incidence of post-operative infections. A retrospective review was undertaken to identify all patients with tibial plateau fractures over a 10-year period (2003–2012), who underwent open reduction internal fixation. MRSA was the most common species [21]. This study demonstrated that most of these pathogens isolated from clinical samples were MDR, and those are potentially enough to destroy the clinical totem pole of a hospital and to precipitate devastating episodes in the community. As analyzed, suppurative infections are one of the major problems of health, as MDR bacteria could attack several organs such as lungs, heart, and kidneys, through BSI [22,23].

CONCLUSION

This surveillance was undertaken for a revision of the antimicrobial stewardship program especially for surgical episodes; the rising concern from frequent SSIs reports in patients attending the orthopedic department with a newer prophylaxis module. A revised antimicrobial stewardship program would reduce nosocomial spread of virulent strains of bacteria, as well as morbidity including the cost of hospitalization. MRSA and *P. aeruginosa* were leading causatives of post-operative infection in orthopedic wounds. Antimicrobial treatment should be revised in empiric coverage for surgical wounds, in view of shenanigans of both pathogens.

ACKNOWLEDGMENTS

This work, R.N. Padhy (RNP) is PI; M. P. Mishra and S. N. Rath were supported as JRFs by a major research project on "Development of standardized herbal extracts against urinary tract bacterial infection" (Grant no. BT/PR8214/PBD/17/863/2013), from the Department of Biotechnology, Government of India, New Delhi.

AUTHORS CONTRIBUTION

PBD, conducted the clinical study, MPM and SNR helped PBD in microbiological study, RNP directed the work holistically in which PBD and SNR wrote the draft copy of the paper. All authors approved the manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest.

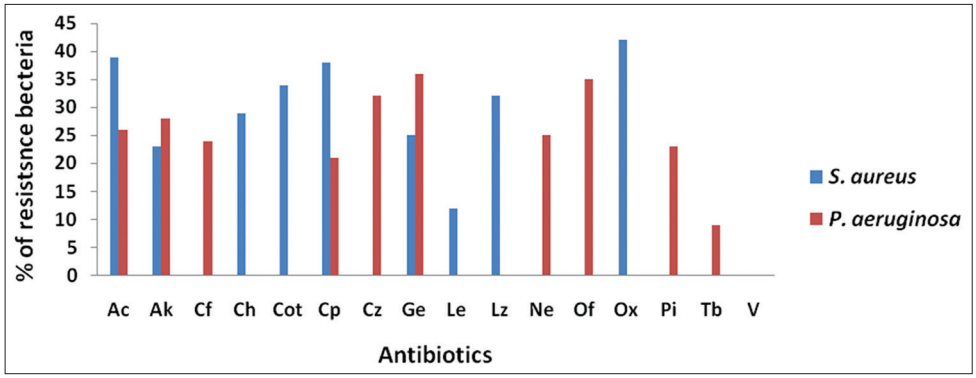

Fig. 3: Antibiogram of resistance bacteria, *Pseudomonas aeruginosa* and *S. aureus*. Antibiotic in µg/disc: Ac: Amikacin 30, Ak: Amoxyclav 30, Cf: Ceftriaxone 30, Ch: chloramphenicol 30, Cp: Ciprofloxacin 5, Cot: Cotrimoxazole 25, Cz: Ceftazidime 30, Ge: Gentamicin 10, Le: Levofloxacin 5, Lz: Linezolid 30, Ne: Netilmicin 30, Of: Ofloxacin 5, Ox: Oxacillin 1, Pi: Piperacillin 100, Tb: Tobramycin 10, V: Vancomycin 30

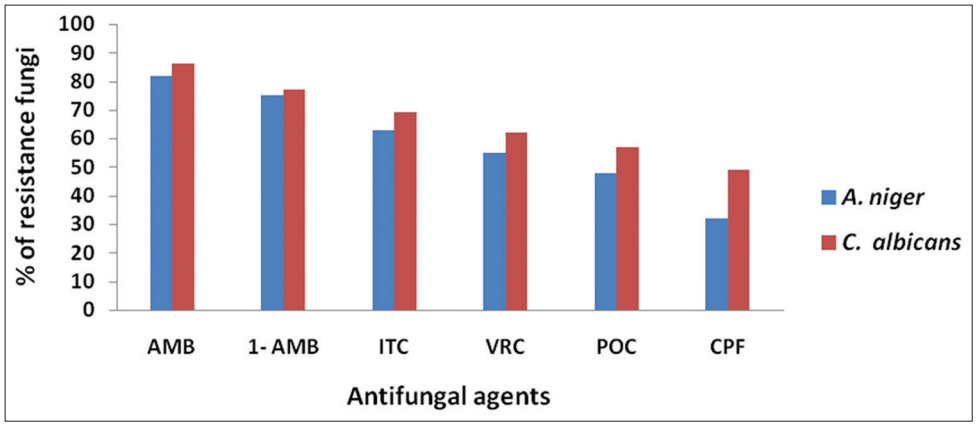

Fig. 4: Antifungal agents used against fungi, *Aspergillus niger* and *Candida albicans*. Antifungal agents: AMB: Amphotericin B; l-AMB: Liposomal AMB, ITC: Itraconazole, VRC: Voriconazole, POS: Posaconazole, CPF: Caspofungin

REFERENCES

1. WHO. Prevention of Hospital Acquired Infections: A Practical Guide. Malta: Department of Communicable Disease, Surveillance and Response; 2002. Available from: http://www.who.int/csr/resources/publications/whocdscsreph200212.pdf. [Last accessed on 2010 Jul 20].
2. Endalafer N, Gebre-Selassie S, Kotisso B. Nosocomial bacterial infections in a tertiary hospital in Ethiopia. J Infect Prev 2011;12:38-43.
3. Napolitano MN. Perspectives in surgical infections: What does the Future hold? Surg Infect 2010; 11:111-23.
4. Datta R, Huang SS. Risk of infection and death due to methicillin-resistant *Staphylococcus aureus* in long-term carriers. Clin Infect Dis 2008;47:176-81.
5. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: Analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004;39:309-17.
6. Nguyen D, MacLeod WB, Cam PD, Cong QT. Incidence and predictors of surgical-site infections in vietnam. Infect Control Hosp Epidemiol 2001;22:485-93.
7. Whitehouse JD, Friedman ND, Kirkland KB, Richardson WJ, Sexton DJ. The impact of surgical-site infections following orthopedic surgery at a community hospital and a university hospital: Adverse quality of life, excess length of stay, and extra cost. Infect Control Hosp Epidemiol 2002;23:183-9.
8. Pittet D. Compliance with hand disinfection and its impact on hospital-acquired infections. J Hosp Infect 2001;48 Suppl A: S40-6.
9. Tammelin A, Ljungqvist B, Reinm¨uller B. Comparison of three distinct surgical clothing systems for protection from air-borne bacteria: a prospective observational study. Patient Saf Surg 2012;15:23.
10. Dubey D, Rath S, Sahu MC, Patnaik L, Debata NK, Padhy RN. Surveillance of infection status of drug resistant *Staphylococcus aureus* in an Indian teaching hospital. Asian Pacif J Trop Dis 2013;3:133-42.
11. Rath S, Padhy RN. Surveillance of acute community acquired urinary tract bacterial infections. J Acute Dis 2015;3:186-95.
12. Mishra MP, Debata NK, Padhy RN. Surveillance of multidrug resistant uropathogenic bacteria in hospitalized patients in Indian. Asian Pac J Trop Biomed 2013;3:315-24.
13. Rath S, Panda M, Sahu MC, Padhy RN. Bayesian analysis of two diagnostic methods for paediatric ringworm infections in a teaching hospital. J Mycol Med 2015;25:191-9.
14. Starčević S, Munitlak S, Mijović B, Mikić D, Suljagić V. Surgical site infection surveillance in orthopedic patients in the military medical academy, belgrade. Vojnosanit Pregl 2015;72:499-504.
15. Lilani SP, Jangale N, Chowdhary A, Daver GB. Surgical site infection in clean and clean-contaminated cases. Indian J Med Microbiol 2005;23:249-52.
16. Patel SM, Patel MH, Patel SD, Soni ST, Kinariwala DM, Vegad MM. Surgical site infections: Incidence and risk factors in a tertiary care hospital, Western India. Nat J Community Med 2012;3:193-6.
17. Mahesh CB, Shivakumar S, Suresh BS, Chidanand SP, Vishwanath Y. A prospective study of surgical site infections in a teaching hospital. J Clin Diagn Res 2010;4:114-9.
18. Malik S, Gupta A, Singh KP, Agarwal J, Singh M. Antibiogram of aerobic bacterial isolates from post-operative wound infections at a tertiary care hospital in India. J Infect Dis Antimicrob Agents 2011;28:45-52.
19. Al-Mulhim FA, Baragbah MA, Sadat-Ali M, Alomran AS, Azam MQ. Prevalence of surgical site infection in orthopedic surgery: A 5-year analysis. Int Surg 2014;99:264-8.
20. Uçkay I, Pittet D, Vaudaux P, Sax H, Lew D, Waldvogel F, *et al.* Foreign body infections due to *Staphylococcus epidermidis*. Ann Med

2009;41:109-19.

21. Momaya AM, Hlavacek J, Etier B, Johannesmeyer D, Oladeji LO, Niemeier TE, *et al.* Risk factors for infection after operative fixation of tibial plateau fractures. Injury 2016;47:1501-5.
22. Techaoei S, Eakwaropas P, Khemjira J, Warachate K. Structure characterization and evaluation potential of antimicrobial. Extracts from *Phellinus linteus* against skin infectious pathogens, *Staphylococcus epidermidis* atcc12228 and *Propionibacterium acnes* dmst14916. *Int J Pharm Sci* 2017;9:70-81.
23. Penta J, Jannu K, Musthyala R. Antimicrobial studies of selected antibiotics and their combination with enzymes. *Int J Pharm Sci* 2010;2:43-4.