INTRODUCTION

An acquired infection in hospital stay by a patient admitted for any health issue is defined as nosocomial or hospital-acquired infection (HAI) [1]. In general, HAI’s are involved with surgical wounds, infection of urinary, respiratory tracts and soft tissues, giving rise to bloodstream infection (BSI), when uncontrolled. Those might be grave enough to surgical site infections (SSIs), leading to morbidity, functional disability, emotional suffering, longer hospitalization, and mortality [2-4]. These constitute major public health problems, promoting unwanted issues of “disability-adjusted life years.” Moreover, SSIs accounting to 17% healthcare-associated infections are the second most common HAIs, next to urinary tract infection [5]. Indeed in orthopedic SSIs, cases of trauma, emergency surgery including dirty wound redressal were associated, and the later was a significant predictor of SSIs [6]. Other causes of SSIs have been identified as the lack of personal cleanliness of patients and indurate attitude of surgeons and paramedical staff toward stringent antiseptic maintenance of the total environment in operation theaters basically. Moreover, device associated [7] and hand washing and associated impeccable habits in maintaining basic hygiene. HAIs are often the underlying cause [8]. However, prophylactic antibiotics are given at the proper time at correct strength/dose and the use of clean surgical clothing or the check of flow of staff into operating room contribute to lowering the incidence of infections [9].

The commensal turned bacterium, Staphylococcus aureus is most prevalent in orthopedic SSIs. Particularly, several clonal variants of S. aureus were resistant to the penicillin group of antibiotics, after which methicillin-resistant was identified as the control. Subsequently, methicillin-resistant S. aureus (MRSA), causing SSIs with/without the emergence of wounds [10]. The most gruesome situation is emergence of MRSA strains with concomitant resistance to most commonly used antibiotics of groups, aminoglycosides, macrolides, fluoroquinolones, chloramphenicol, tetracycline, cephalosporins and other β-lactams, amoxicillin-clavulanic acid, piperacillin-tazobactam carbapenem, and imipenem, as SSIs.

Moreover, Pseudomonas aeruginosa had been seen as a notorious pathogen in this hospital too [11]. These pathogens are mainly found in wounds and urinary tract but lead to inner cells causing septicemia and associated comorbidities, through BSI. As it is, the rate of invasion of a pathogenic bacterium directly depends on the level of drug resistance, apart from the challenged immune condition of patients.

This work describes surveillance of bacterial flora from wound sample of patients attending the orthopedic department of the hospital, over a period of 18 months. Two fungi, Aspergillus niger and Candida albicans were isolated along with bacteria. This surveillance was undertaken for a revision of the antimicrobial stewardship program; the rising concern from frequent SSIs reports in patients attending the orthopedic department with a newer prophylaxis module. Revised antimicrobial stewardship program would reduce nosocomial spread of virulent strains of bacteria, as well as morbidity including the cost of hospitalization.

METHODS

The present study was done between August 2015 and January 2017 in this hospital. Inclusion criteria were all closed fractures admitted to orthopedics department in this hospital. A total of 621 swabs were taken from the surgical site after definitive treatment of the fracture. Swabs were cultured on blood and MacConkey agar plates that were
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, ceftaxidime, gentamicin, piperacillin, netilmycin, 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 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 (9). Fungi accounted for 13 isolates of A. niger and 28 isolates of C. albicans from 509 growth-yielding samples (Table 1).

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/disc had 91%, followed by ciprofloxacin 5 µg/disc 79% and piperacillin 100 µg/disc 77% and 100% S. aureus isolates were susceptible to vancomycin 30 µg/disc, followed by 88% to levofloxacin 5 µg/disc and 77% isolates to amoxiclav 30 µg/disc. 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 lipidosomal 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 lipidosomal 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

| 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) |

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

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

| Antibiotics | S. aureus | P. aeruginosa |
|------------|-----------|--------------|
| Ac | 39 | 26 |
| Ak | 23 | 28 |
| C | 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, C: Ceftriaxone 30, Ch: Chloramphenicol 30, Cp: Ciprofloxacin 5, Cot: Cotrimoxazole 25, Cz: Cefazidime 30, Ge: Gentamicin 10, Lz: Linezolid 30, Ne: Netilmycin 30, Of: Ofloxacine 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.
S. aureus was predominant in surgical sites, as the most frequently isolated pathogen from SSIs. Isolated pathogens were considered, accordingly for infection control measures aggressively, screening protocols for colonization of these pathogens should be revised in empiric coverage for surgical wounds, in view of 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. 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.

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.

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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.

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, 1-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 MelleSa chromogenic agar

Fig. 2: Candida albicans in potato dextrose agar media
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