examined for the growth of bacteria. All positive cultures were identified by their characteristic appearance on their respective media, Gram staining reaction and confirmed by the pattern of biochemical reactions. [14] If no growth was observed on the plates, subcultures were made from the Brain Heart infusion broth onto 5% sheep blood agar and MacConkey agar, which were observed after 24 hours of incubation. Antimicrobial susceptibility testing done on Mueller Hinton Agar by Kirby Bauer disc diffusion method as per CLSI guidelines.[15]

All the confirmed *Staphylococcus aureus* and coagulase-negative *Staphylococcus spp* (CONS) strains were subsequently screened for Methicillin resistance based on Kirby-Bauer disk diffusion method using cefoxitin discs (30 µg) obtained from Hi-Media Laboratories Pvt. Ltd.

3. Interpretation

The isolates were considered Methicillin Resistant *Staphylococcus aureus* (MRSA) if the zone of inhibition was less than 21 mm and Methicillin Sensitive *Staphylococcus aureus* (MSSA) if it was ≥ 22 mm. [15] For coagulase-negative *Staphylococcus* (CoNS), if the zone of inhibition was less than 24 mm considered as Methicillin resistant coagulase-negative *Staphylococcus* (MRCONS) and if it was ≥ 25 mm Methicillin sensitive coagulase-negative *Staphylococcus* (MSCONS). [15]

The antibiotics tested against *Staphylococcus spp* were penicillin-G, cephalexin, cefazolin, erythromycin, clindamycin, gentamicin, amikacin, vancomycin, teicoplanin, linezolid, rifampicin and chloramphenicol. The following antibiotics were used for Gram Negative bacilli: ampicillin, cephalexin; ceftriaxone; cefotaxime; amoxicillin-clavulanate; ciprofloxacin; gentamicin; amikacin; imipenem; meropenem; piperacillin-tazobactam and the antibiotics tested against *Pseudomonas spp* were gentamicin, amikacin, ciprofloxacin, aztreonam, ceftazidime, piperacillin-tazobactam, imipenem, meropenem, netilmicin and tobramycin. The tests were interpreted as Sensitive, Intermediate susceptible or Resistant in accordance with standard recommendation. [15]

4. Statistical Analysis

Data was entered into a computerized Excel (Microsoft Excel 2009) spread sheet, and subsequently it was analyzed using SPSS (trial version 20) software. Descriptive statistics (means and percentages) were used wherever necessary.

5. Results

During the one year study period, a total of 195 specimens were received from orthopedic department which included specimens from open fractures (16/88), surgical site infection (41/88), deep bed sores involving bones and miscellaneous ones(31/88). 45.13% (88/195) specimens showed culture positivity and 54.88% (107/195) specimens did not show any growth. The age of patients ranged from 1 year to 75 years with a mean age of 39 years.

In our study, both Gram positive and Gram negative organisms were isolated in equal numbers (44/88). The common isolates found in our study are *Staphylococcus aureus* , *Escherichia coli* , *Pseudomonas aeruginosa* , *Klebsiella Spp* , *Non fermenting gram negative bacilli* and *Proteus spp.* Majority of the organisms isolated were from surgical site infections (46.59%) followed by wounds (35.22%) and open fracture (18.18%). The incidence of various microbes in relation to orthopedic illness and procedures are detailed in the table 1. The antibiotic susceptibility pattern of gram positive organisms and gram negative organisms are detailed in the table 2 and 3 respectively.

6. Discussion

Infections are known to occur in spite of aseptic precautions by the orthopedicians. Eighty eight (45.13%) samples showed positive culture in our study which coincides with Abraham Y et al [16] study which showed 41% positivity, whereas Gomez et al [17] and Zimmeli et al [18] reported positive cultures in 60% and 89% respectively.

In 1950's and 60's *Staphylococcus aureus* used to be the most common strain. [3] In late 70's there was a shift from gram positive infections to gram negative infections among orthopedic patients. [19, 20]

Staphylococcus aureus (40.90% of the total number of isolates) is the most common organism isolated from our study. Anterior nares, palm acts as important reservoirs for *Staphylococcus aureus*, about 10-20% of the healthy individuals will arbores this organism. Bed sheets, instruments and dressings have been found to act as reservoirs. Bergqvist et al [21] and Dan et al [22] found that 29.8% of hospitalized patients and 26.6% of hospital staff respectively are carriers. 12.5% (11/88) of our isolates are Methicillin Resistant *Staphylococcus aureus*. Other studies have observed MRSA ranging from 5.6% to 37.9 %,[23, 24] thus indicating lower range of prevalence of MRSA during this study period.

Escherichia coli is the second most common pathogen (15.9% cases), especially in SSI and patients with wound infection. *Escherichia coli* is a commensal of gut and as many patients are admitted for prolonged periods, contamination of wounds, dressings, linen, clothes and hands during perineal hygiene plays a major role in increasing chances of transmission of infection.

Pseudomonas aeruginosa (13.6% cases) is the third most common cause, which commonly isolated from SSI and bed sores. Pseudomonas can multiply on common objects in a hospital environments such as dressings materials, buckets used for soaking Plaster of paris bandages and foreceps, has been documented by Agarwal at al [25] and Dade and Hall.[26] *Klebsiella Spp* which has also been isolated in a significant number (10.2% cases) in our study. Other gram negative organisms like NFGNB (5.7% cases), Proteus spp. (4.6% cases) isolated from SSI.

In our study, all the MRSA (12.5%) isolates were sensitive to vancomycin, linezolid and teicoplanin. Many other studies have reported all the staphylococcal isolates being sensitive to vancomycin and linezolid.[27] Currently vancomycin resistance *Staphylococcus aureus* (VRSA) is not widespread. Vancomycin remains the first choice of treatment for MRSA. There was good sensitivity of MRSA for doxycycline (90.90%), and clindamycin (63.63%), so these drugs are also useful for SSI by MRSA. Among coagulase negative staphylococcus (5.9%) isolates only 2.3% of isolates were methicillin resistant, which were sensitive to vancomycin, teicoplanin and linezoild.

In this study, *E.coli* showed more resistance to ampicillin (93%), piperacillin (93%), cephelaxin (93%), cefuroxime (86%), amoxicillin/clavulanic acid (79%), ciprofloxacin (71%), cefotaxime (72%), and less resistance to ceftazidime (57%), piperacillin/tazobactum (30%), gentamicin (29%), meropenem (14%) and imipenem (7%), while amikacin, colistin and tigecycline were 100% sensitive (Table No.3). Similar finding were observed by Aratikalakutakar, Vishwanath LYemul.[28] 36% ESBL *E.coli* were isolated.

Pseudomonas aeruginosa showed 50% resistant to piperacillin, ciprofloxacin and cotrimoxazole, 33% to ceftazidime and cefepime, 25% to gentamicin and netilmicin, 17% to piperacillin/tazobactum and meropenem, while tobramyin, imipenem, colistin were 100% sensitive (Table No.3). Similar observation was reported by Aratikalakutakar, Vishwanath LYemul.[28]

From our results, we observed that amoxicillin/clavulanic acid , ceftriaxone and ceftazidime cannot be recommended for use as an empirical therapy in SSI and open fracture infections because these drugs were inactive against most strains. Based on the antimicrobial susceptibility data, we suggest that piperacillin/tazobactum and imipenem are the most effective agents against most of gram negative bacteria and doxyciline, vancomycin, linezolid are the most effective agents against gram positive organisms. Colistin and tigecyclin showed 100% sensitivity by all gram negative bacteria, but these drugs are kept as reserve, should be used judiciously.

7. Conclusion

High rates of antibiotic resistance were observed in our study, due to widespread usage of broad spectrum antibiotics. While deciding antibiotic therapy many factors must be considered like previous antibiotic history, knowledge of most common causative organism in these infections, and their antibiotic profile. By multidisciplinary collaboration involving: the orthopedic surgeons, infectious disease specialist and clinical microbiologist we can further reduce the incidence of infection in our hospital.

8. Recommendations

There is a need for formulation of antibiotic policy in tandem with clinicians/orthopedicians and antibiotic sensitivity pattern. A strict adherence to the antibiotic policy and formulary restriction is a must.

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Table 1: Frequency of Gram positive and gram negative organisms isolated from different sites.

Organism	Open Fracture	SSI	Bedsore	Total
Gram positive organisms				
Staphylococcus aureus (MSSA)	7	5	13	25 (28.4)
MRSA	5	3	3	11(12.5)
Streptococcus spp	0	2	0	2 (2.3)
Enterococcus Spp	0	1	0	1 (1.2)
Coagulase negative Staphylococcus (MSCONS)	0	2	1	3 (3.4)
MRCONS	0	2	0	2 (2.3)
Gram negative organisms				
Escherichia coli	1	6	7	14 (15.9)
Pseudomonas aeruginosa	0	6	6	12 (13.6)
Klebsiella pneumonia	2	5	0	7 (7.9)
Non-fermenting Gram negative rods	1	3	1	5 (5.7)
Klebsiella oxytoca	0	2	0	2 (2.3)
Proteus mirabilis	0	2	0	2 (2.3)
Proteus vulgaris	0	2	0	2 (2.3)
TOTAL	16 (18.18)	41(46.59)	31(35.22)	88 (100)

Table 2: Antibiotic sensitivity pattern of Gram positive organisms

Oraganism	**Pn**	**AMP**	**AMC**	**FOX**	**LEX**	**CXM**	**ERY**	**CLI**	**CIP**	**DOX**	**SXT**	**CHL**	**GEN**	**VAN**	**LZ**	**TEI**
MSSA (25)	0	0	100	100	100	100	81.81	81.81	63.63	90.90	63.63	45.45	63.63	100	100	100
MRSA (11)	0	0	0	0	0	0	45.45	63.63	45.45	90.90	45.45	27.27	54.54	100	100	100
Streptococcus spp (2)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Enterococcus Spp (1)	0	100	100	-	0	0	100	100	0	100	0	0	0	100	100	100
MSCONS (3)	0	0	100	100	100	100	50	50	50	100	50	0	50	100	100	100
MRCONS (2)	0	0	0	0	0	0	50	50	0	100	50	0	50	100	100	100

Table 3: Antibiotic sensitivity pattern of Gram negative organisms

Organism	*AMP*	*PIP*	*AMC*	*LEX*	*CXM*	*CTX*	*CAZ*	*CRO*	*FEP*	*PIT*	*ATM*	*ETP*	*IMP*	*MEM*	*GEN*	*AMK*	*NET*	*TOB*	*CIP*	*SXT*	*COL*	*TGC*
E.coli (14)	7	7	21	7	14	28	43	50	77	70	70	57	93	86	71	100	-	-	29	69	100	100
P.aeruginosa (12)	-	50	-	-	-	-	67	-	67	83	83		100	83	75	92	75	100	50	50	100	100
K. pneumonia (7)	0	14	57	29	29	57	57	57	71	86	71	86	100	86	86	86	-	-	86	86	100	100
NFGNB (5)	-	0	-	-	-	-	20	-	20	20	20	40	40	40	20	20	40	60	0	20	100	100
K. oxytoca (2)	0	0	0	0	0	50	0	0	100	100	50	100	100	100	100	100	-	-	50	50	100	100
P.mirabilis (2)	50	50	50	50	50	50	50	50	100	100	50	100	100	100	100	100	-	-	100	100	100	100
P. vulgaris (2)	0	0	0	0	0	50	50	50	100	100	50	100	100	100	50	100	-	-	50	100	100	100

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