

# Prevalence of Multidrug Resistant Organisms in Wound Infections

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## ABSTRACT

The objectives of this study are to review the incidence of pathogens causing wound infections from 3,693 wound pus aspirates/swab samples obtained from Orthopaedic and Surgical Unit in a tertiary care hospital in India from August 2000 to July 2001. The antimicrobial profile to the pathogens was reviewed and included antimicrobial susceptibility, multidrug resistance (MDR) and minimum inhibitory concentrations (MIC). One thousand six hundred and seventy-two (45.3%) samples were sterile. Common isolates were *Staphylococcus aureus* (32.3%), *Klebsiella* spp. (22.0%), *Pseudomonas aeruginosa* (18.7%) and *Escherichia coli* (17.4%). Highest MDR was found in *P. aeruginosa* at 54.8 percent, followed by *Acinetobacter* spp. (40.0%), *Klebsiella* spp. (33.3%) and *Citrobacter* (33.3%). Amikacin and ceftizoxime showed higher sensitivity than other drugs to *E. coli*, *Klebsiella* spp. and *P. aeruginosa*. Clindamycin and amikacin showed high sensitivity to *S. aureus*. Large numbers of various isolates showed very high MIC values. Selection of antibiotics is required according to local MDR and sensitivity to organisms. Every hospital should formulate a local antibiotic policy for infection control so that optimal antibiotics could be initiated without delay. (*J Infect Dis Antimicrob Agents* 2002;19:111-7.)

## INTRODUCTION

Inadequate antimicrobial treatment, defined as ineffective treatment of an infection, is an important factor in the emergence of antibiotic resistant bacteria.

Factors that contribute to inadequate antimicrobial treatment of hospitalized patients include the prior antibiotic use, broad spectrum antibiotics, prolonged hospital stay and the presence of invasive medical

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devices. Other factors include the spread of resistant organisms through overcrowding and inadequate hospital infection control practices.<sup>1</sup> There is a concern that certain bacterial infections may become untreatable. Appropriate use of antibiotics is very important. This study looks at wound infections and reviews the prevalence of MDR organisms, antibiotic susceptibility pattern and MIC, to provide guidelines on appropriate treatment and ensure infection control policies, leading to appropriate treatment of wound infections and prevention of emergence of antimicrobial resistance.

### MATERIALS AND METHODS

**Criteria for wound selection:** Our study included patients with acute and chronic wounds with purulent discharge or painful spreading erythema around a wound. This included cutaneous abscesses, traumatic wounds, foot ulcers and pressure ulcers. Patients included were from Surgical and Orthopedic Units of Pt. B.D. Sharma Post Graduate Institute of Medical Sciences in Rohtak, a large tertiary level hospital (India) from August 2000 to July 2001.

There were 3,693 specimens; 3,400 were pus aspirates, and in 293 specimens, there was no frank pus but the wound was swabbed. Samples were transported to the Microbiology Laboratory and plated onto the 5 percent sheep blood agar and MacConkey's medium (Hi Media) and incubated at 37°C for 24 hours with 5-10 percent CO<sub>2</sub> aerobically and the microorganisms were identified by standard microbiological methods. Sensitivity testing was carried out in only predominant pathogenic organisms.

Antimicrobial susceptibility of clinical isolates was determined by Stokes technique.<sup>2</sup> Susceptibility testing of coliforms and *Proteus* spp. used discs with tetracycline (10 mcg), cefuroxime (30 mcg), ampicillin (10 mcg), gentamicin (10 mcg), co-trimoxazole (25 mcg), norfloxacin (10 mcg), amikacin (10 mcg), cefotaxime (10 mcg), ciprofloxacin (10 mcg), ceftizoxime (30 mcg). Susceptibility of *P. aeruginosa* used discs with gentamicin (10 mcg), amikacin (10 mcg), ciprofloxacin (10 mcg), ceftazidime (10 mcg), piperacillin (100 mcg)

and ceftizoxime (30 mcg). Susceptibility of *S. aureus* was determined to penicillin (10 mcg), tetracycline (10 mcg), erythromycin (15 mcg), co-trimoxazole (25 mcg), cefuroxime (30 mcg), amikacin (10 mcg), and clindamycin (2 mcg). The interpretation of inhibition zone sizes was done by National Committee for Clinical Laboratory Standards (NCCLS) criteria.<sup>3</sup>

The MIC test on multiresistant (resistant to  $\geq 3$  antimicrobials) organisms used an organism purely grown on primary plating. The agar dilution method for MICs were determined in 30 MDR isolates of *E. coli*, *K. pneumoniae* and *Proteus* spp. for amikacin, ciprofloxacin, cefotaxime and ceftizoxime. MICs for *P. aeruginosa* were tested against ciprofloxacin, ofloxacin, cefotaxime, amikacin and ceftizoxime. The MICs for *S. aureus* were determined for erythromycin, clindamycin, oxacillin and cefuroxime. Inocula were adjusted to yield approximately 10<sup>4</sup> cfu/spot on MHA and plates were incubated aerobically at 37°C × 18 hours. The MIC was considered to be the lowest concentration that prevented visible growth or yielded < 6 discrete colonies. Three quality control strains used in study were *S. aureus* NCTC 6571, *E. coli* NCTC 10418 and *P. aeruginosa* NCTC 10662. Break points for susceptibility were taken from NCCLS.<sup>3</sup>

### RESULTS

Out of 3,693 samples processed, 2,021 (54.7%) showed growth while 1,672 (45.3%) were sterile. Of 2,021 isolates, 532 (26.3%) showed more than one microorganisms while 1,489 isolates showed growth of one organism. The commonest isolates were *S. aureus* (32.3%), *Klebsiella* spp. (22.0%), *P. aeruginosa* (18.7%), *E. coli* (17.4%) and *Proteus* spp. (1.6%). Seventy percent of *Klebsiella* spp. were *K. pneumoniae* and 30 percent were *K. oxytoca*. Of *Proteus* spp., 60 percent were *P. vulgaris* and 40 percent were *P. mirabilis*. By Stoke's disk diffusion method sensitivity of *S. aureus* was maximal to amikacin (65.9%) and clindamycin (65.1%). *P. aeruginosa* was maximally sensitive to amikacin (31.8%) and ceftizoxime (26.5%). Sensitivity of *E. coli* was maximum to amikacin (69.2%)

and ceftizoxime (59.2%) while *Klebsiella* spp. showed maximum sensitivity to amikacin (47.3%) and ceftizoxime (37.1%) (Table 1).

Multidrug resistance to 3 or more drugs and resistance of microorganisms to all the antibiotics is shown in Table 2. Resistance of microorganisms to all tested antibiotics was highest in *P. aeruginosa* (54.8%) followed by *Acinetobacter* spp. (40.0%), *Klebsiella*

(33.3%), *Citrobacter* (33.3%) *Proteus* spp. (29.0%), *E. coli* (21.5%) and *S. aureus* (10.5%). *Streptococcus pyogenes* was uniformly sensitive to all antibiotics including penicillin.

MIC results of 30 isolates each of *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *E. coli* and *Proteus* spp. are shown in Table 3, 4, 5, 6, 7 respectively. Sensitivity of various isolates based on MICs is shown in Table 8.

**Table 1. Percentage of sensitivity of various isolates in surgical wounds by Stoke's disc diffusion method.**

Drug	<i>S.aureus</i>	<i>K.pneumoniae</i>	<i>P.aeruginosa</i>	<i>E.coli</i>	<i>Proteus</i> spp.	<i>Acinetobacter</i> spp.	<i>Enterobacter</i> spp.	<i>Citrobacter</i> spp.
Amikacin (10 mcg)	65.9%	47.3%	31.8%	69.2%	20.0%	50.0%	83.3%	33.3%
Ampicillin (10 mcg)	-	5.3%	-	3.5%	4.0%	10.0%	0.0%	0.0%
Cefotaxime (10 mcg)	-	20.6%	-	17.3%	36.0%	30.0%	83.3%	33.3%
Ceftazidime (10 mcg)	-	-	12.1%	-	-	-	-	-
Ceftizoxime (30 mcg)	-	37.1%	26.5%	59.2%	44.0%	50.0%	50.0%	50.0%
Cefuroxime (30 mcg)	42.1%	10.0%	-	9.6%	12.0%	20.0%	50.0%	33.3%
Clindamycin (2 mcg)	65.1%	-	-	-	-	-	-	-
Co-trimoxazole (25 mcg)	10.3%	4.4%	-	4.2%	0.0%	0.0%	0.0%	0.0%
Ciprofloxacin (10 mcg)	-	22.7%	18.6%	12.3%	20.0%	40.0%	50.0%	33.3%
Erythromycin (15 mcg)	45.8	-	-	-	-	-	-	-
Gentamicin (10 mcg)	-	14.6%	10.0%	20.4%	12.0%	0.0%	33.3%	0.0%
Norfloxacin (10 mcg)	-	15.0%	-	8.1%	12.0%	10.0%	50.0%	33.3%
Penicillin (10 mcg)	28.0%	-	-	-	-	-	-	-
Piperacillin (10 mcg)	-	-	8.6%	-	-	-	-	-
Tetracycline (10 mcg)	40.4%	12.5%	-	9.6%	8.0%	10.0%	16.6%	0.0%

**Table 2. Prevalence of various isolates in surgical wounds and multidrug resistance pattern.**

No.	Organism	Number of isolates	≥ 3 drugs (R)	All(R)
1	<i>Staphylococcus aureus</i>	482	331	51 (10.5%)
2	<i>Pseudomonas aeruginosa</i>	279	257	153 (54.8%)
3	<i>Escherichia coli</i>	260	252	56 (21.5%)
4	<i>Klebsiella</i> spp.	329	307	107 (33.3%)
5	<i>Proteus</i> spp.	24	22	7 (29.0%)
6	<i>Streptococcus pyogenes</i>	13	0	0
7	<i>Acinetobacter</i> spp.	10	9	4 (40.0%)
8	<i>Enterobacter</i> spp.	6	5	0
9	<i>Citrobacter</i> spp.	6	6	2 (33.3%)

**Table 3. MICs of *S. aureus* against various antimicrobial agents.**

Break point MIC (mcg/ml)	Erythromycin ≥ 16	Clindamycin ≥ 4	Oxacillin ≥ 4	Cefuroxime ≥ 32
≥ 128	7	-	-	6
64	4	-	-	3
32	1	-	-	0
16	0	5	9	0
8	0	0	2	0
4	18	1	0	21
2	-	0	0	-
1	-	0	1	-
0.5	-	24	18	-

**Table 4. MICs of *K. pneumoniae* against various antimicrobial agents.**

Break point MIC (mcg/ml)	Amikacin ≥ 32	Ciprofloxacin ≥ 4	Cefotaxime ≥ 64	Ceftizoxime ≥ 32
≥ 128	8	-	5	3
64	5	-	1	1
32	1	10	4	2
16	6	2	2	1
8	2	1	3	8
4	8	1	15	15
2	-	5	-	-
1	-	11	-	-

Table 5. MICs of *Pseudomonas* spp. against various antimicrobial agents.

Break point MIC (mcg/ml)	Amikacin $\geq 32$	Ciprofloxacin $\geq 4$	Ofloxacin $\geq 8$	Cefotaxime $\geq 64$	Ceftizoxime $\geq 32$
$\geq 128$	17	-	-	17	18
64	0	-	-	7	2
32	3	19	22	5	1
16	5	1	0	0	8
8	4	0	0	0	1
4	1	0	0	1	0
2	-	0	8	-	-
1	-	10	0	-	-

Table 6. MICs of *E. coli* against various antimicrobial agents.

Break point MIC (mcg/ml)	Amikacin $\geq 32$	Ciprofloxacin $\geq 4$	Cefotaxime $\geq 64$	Ceftizoxime $\geq 32$
$\geq 128$	9	-	5	2
64	3	-	5	2
32	4	12	3	3
16	9	1	0	0
8	0	2	0	0
4	5	0	17	23
2	-	2	-	-
1	-	13	-	-

Table 7. MICs of *Proteus* spp. against various antimicrobial agents.

Break point MIC (mcg/ml)	Gentamicin $\geq 8$	Amikacin $\geq 32$	Ciprofloxacin $\geq 4$	Cefotaxime $\geq 64$	Ceftizoxime $\geq 32$
> 128	20	15	-	4	2
64	0	1	-	2	8
32	2	3	16	6	5
16	2	4	2	3	4
8	3	2	2	5	4
4	3	5	1	10	7
2	-	-	0	-	-
1	-	-	9	-	-

Table 8. Sensitivity of various isolates based on MICs.

Drug	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>Proteus</i> spp.
Amikacin	-	53.3%	33.3%	46.6%	36.6%
Cefotaxime	-	80.0%	20.0%	66.6%	73.3%
Ceftizoxime	-	80.0%	30.0%	76.6%	50.0%
Cefuroxime	70.0%	-	-	-	-
Clindamycin	80.0%	-	-	-	-
Ciprofloxacin	-	53.3%	33.3%	50.0%	30.0%
Erythromycin	60.0%	-	-	-	-
Gentamicin	-	-	-	-	10.0%
Ofloxacin	-	-	26.6%	-	-
Oxacillin	63.3%	-	-	-	-

## DISCUSSION

Microbiology laboratory plays important role in identification of one or more microorganisms and which microorganism should be assayed for antibiotic susceptibility. MDR organisms in wound infections lead to failure to heal, increase treatment cost and there is a prolonged wound management practice.<sup>4</sup> The hospital environment is the main focus for the emergence and dissemination of antibiotic resistant bacteria.<sup>5</sup>

In the present study the most common wound isolate was *S. aureus* (32.3%) followed by *K. pneumoniae* (22.0%), *Pseudomonas* spp. (18.7%) and *E. coli* (17.4%). Twenty years ago, most wound infections were due to coagulase positive *S. aureus* but over the last 5 years, the percentage of gram-negative bacilli has increased.<sup>6</sup> The present study also shows that gram-negative bacilli are the principal organisms in wound infections.

Selective pressure of the antimicrobial agents is a major factor in the emergence of resistance among bacterial pathogens.<sup>7</sup> In the present study MDR was noted among all predominant pathogens in orthopedics and surgical wards. MDR to  $\geq 3$  drugs was present in > 90 percent of isolates and many of these isolates were even resistant to all the antibiotics tested by disk diffusion method. *Pseudomonas* spp. which were resistant to all antibiotics were 54.8 percent followed by *Acinetobacter* (40.0%), and *K. pneumoniae*

(33.3%), *Citrobacter* (33.3%), *Proteus* spp. (29.9%), *E. coli* (21.5%) and *S. aureus* (10.5%). These results leave us with problematic therapy of such MDR organisms. Also there is need to control the dissemination of potentially pathogenic members of the wound microflora into the surrounding environment in order to minimize the opportunity for cross-infection.<sup>4</sup>

MDR organisms have very high MIC values for various commonly used antibiotics. Our MIC studies show that clindamycin (80%) and cefuroxime (70%) are more effective in *S. aureus* isolates on the other hand rate of oxacillin resistant *S. aureus* (ORSA) was 36.6 percent in our hospital among MDR strains selected for MIC study. Amikacin, quinolones and newer cephalosporins are the only effective antimicrobial agents for treatment of infections caused by gram-negative organisms. Specifically, third generation cephalosporins i.e. cefotaxime and ceftizoxime work better in wound infection caused by MDR isolates of *Klebsiella* spp., *E. coli* and *Proteus* spp. However, *Pseudomonas* isolates show poor sensitivity even for antibiotics like amikacin (33%), ciprofloxacin (33%), ceftizoxime (30%).

Continuous monitoring of bacterial resistance is very important and should be undertaken by all laboratories to ensure that local and empirical treatment policies remain accurate and reliable.<sup>1</sup> It is important to have a database of microbial antibiotic sensitivity for

various diseases in community and hospital settings so that empirical treatment with optimal antibiotics can be initiated without delay. It is important to disseminate the data on trends of antimicrobial susceptibility and resistance locally, nationally and internationally. This will help us to maintain the efficacy of antibiotics by determining which diseases they can eradicate and which organisms are still susceptible, thus preventing therapeutic failures.

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