

## **Research Article**

# **Prevalence and Antibiotic Sensitivity Pattern of Bacteria Isolated from Nosocomial Infections in Respiratory Intensive Care Unit of a Teaching Tertiary Care Health Facility**

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**Abstract:** In India, nosocomial infections (NIs) in patients admitted to intensive care units are recognized as a significant problem. The rate of NIs is two to five times higher in intensive care units than that of the general inpatient population. The aim of the present study was to know the prevalence and antibiotic susceptibility pattern of bacteria isolated from patients suffering from NIs admitted in Respiratory intensive care unit (RICU) of our hospital. The present study was designed to carry out prospectively for duration of two years (2008-2010). Hundred patients on mechanical ventilation of all age groups and sex admitted to RICU and suffering from NIs were included in the study. Various samples like endotracheal secretions, blood, urine, pus and post-operative wound swabs were collected aseptically and processed. Antibiotic susceptibility pattern of bacterial isolates was tested by Modified Stokes method. Environmental samples from various surfaces were collected periodically to assess the possible reservoir of nosocomial infections. 184 (92.9%) gram-negative and 14 (7%) gram-positive bacterial pathogens were isolated. Most frequently isolated organism was *P. aeruginosa* 48/184 (26%). The second most common organism was *Acinetobacter baumannii* 42/184 (22.8%). Imipenem and meropenem were found to be most effective drugs as 78% and 72.1% gram-negative bacteria and 81.3% and 62.5% of *P. aeruginosa* strains were sensitive to these antibiotics respectively. Majority (98.4%) of the isolates showed resistance to three or more than three drugs. Regular monitoring of the resistance pattern of bacterial pathogens from ICU patients is needed to prescribe appropriate treatment.

**Keywords:** Antimicrobial resistance, Intensive care unit, Mechanical ventilation, Microorganisms, Nosocomial infections, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*.

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## **INTRODUCTION**

Infections that become clinically evident after 48 hours of hospitalisation are considered nosocomial infections (NIs). Patient may develop a nosocomial infection after being discharged from hospital, if the organism apparently was acquired in hospital. The potential impact of nosocomial infections is considerable when assessed in terms of incidence, morbidity, mortality and financial burden. These costs are attributable to extended lengths of hospital stay and additional antibiotic utilization. Nosocomial infections become especially prominent in the intensive care unit (ICU) where the rate is two to five times higher than that of the general inpatient population due to growing complexity of ICU, impaired host defences of patients

in ICU, invasive procedures and monitoring, exposure to multiple antibiotics, frequent contact with health care staff, colonization with resistant microorganisms and cross infections. Common nosocomial infections in ICU include urinary tract infections, chest infections particularly ventilator associated pneumonia (VAP), blood stream infections and surgical site infections [1].

Nosocomial infections are frequently associated with drug-resistant micro-organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) and extended spectrum  $\beta$ -lactamase (ESBL)-producing gram negative bacteria, which can pose considerable therapeutic problems. Organisms isolated from patients in ICU are more likely to be resistant to

antibiotics than those isolated from general-ward patients or outpatients, probably because there is high antimicrobial selection pressure in these individuals [2, 3].

Microbiology laboratories play an important role in treatment and prevention of hospital acquired infections. Culture of blood, urine, sputum, pus, other body fluids or tissues are especially important in order to identify the bacteria causing the infection. On the basis of results of antibiotic susceptibility pattern of the organisms the effective antibiotic therapy is started which may result in decrease in incidence of antimicrobial resistance and prevention of nosocomial infections. Hence, the present study was conducted to know the frequency of NIs and antibiotic sensitivity pattern of bacteria isolated from nosocomial infections in an ICU of our hospital.

## MATERIALS AND METHODS

### Study design and population

The present prospective study was carried out on critically ill patients admitted to Respiratory intensive care unit (RICU) either directly or transferred from other wards of our institute over a period of two years. A total of hundred patients on mechanical ventilation of all age groups and sex admitted to RICU for reasons other than infection were included in the study. Patients in whom signs of respiratory tract infections, bacteraemia, urinary tract infections and surgical site infections appeared at least 48 hours after admission to RICU were included in the study. Infections were diagnosed according to CDC criteria [4]. Patients with infections at the time of admission in RICU were excluded from the study.

### Sample collection and processing:

Various samples like endotracheal secretions, blood, urine, pus and post-operative wound swabs were collected aseptically by standard procedures from all the patients who fulfilled the inclusion criteria for the present study. Environmental samples from floors, beds, walls, furniture and sinks were collected with swabs moistened in glucose broth periodically to assess the possible reservoir of nosocomial infections. Air sampling was done by settle plate method. All the samples were processed by standard Microbiological procedures. Within half an hour of collection, cultures were made on blood agar and MacConkey agar. Isolates obtained from various samples were identified by colony morphology, staining characteristics and biochemical reactions [5].

### Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by Modified Stokes disc diffusion method on Mueller-Hinton agar. *E. coli* NCTC 10418, *P. aeruginosa* NCTC 10662 and *S. aureus* NCTC 6571 were used as control

strains.[6] The antimicrobial discs were procured from Hi-Media Mumbai.

For gram-positive isolates the following antimicrobial discs were put up with disc concentration in parenthesis (mcg=microgram): penicillin (2 units), oxacillin (1mcg), tetracycline (30mcg), erythromycin (15mcg), cefazolin (30mcg), cefuroxime (30mcg), clindamycin (2mcg), linezolid (30mcg), vancomycin (30mcg), norfloxacin (10mcg), ciprofloxacin (5mcg), cotrimoxazole (25mcg), and amoxycillin/ clavulanate (20/10mcg).

For gram negative isolates- ampicillin (10mcg), gentamicin (10mcg), cefuroxime (30mcg), norfloxacin (10mcg), amikacin (30mcg), cefotaxime (30mcg), ciprofloxacin (5mcg), ceftizoxime (30mcg), imipenem (10mcg), aztreonam (30mcg), ceftazidime (30mcg), piperacillin (100mcg), cefazolin (30mcg), amoxicillin/ clavulanate (20/10mcg), cefepime (30mcg), cotrimoxazole (25mcg), meropenem (10mcg) and piperacillin/tazobactam (100/10mcg) were used.

For *Pseudomonas* species- ticarcillin (75mcg), gentamicin (10mcg), cefoperazone (75mcg), cefuroxime (30mcg), norfloxacin (10mcg), amikacin (30mcg), tobramycin (10mcg), cefotaxime (30mcg), netilmicin (30mcg), carbenicillin (100mcg), norfloxacin (10mcg), ceftizoxime (30mcg), imipenem (10mcg), aztreonam (30mcg), ceftazidime (30mcg), piperacillin (100mcg), ceftizoxime (30mcg), cefepime (30mcg), nitrofurantoin (50mcg), meropenem (10mcg) and piperacillin/tazobactam (100/10mcg) were used.

## RESULTS

Out of 100 patients, 67 were male and 33 were female patients. Maximum number of male cases were from 21-30 years age groups 24/67 (35.8%), followed by 11-20 years 13/67 (19.4%) age groups, 31-40 years 12/67 (17.9%), more than 60 years 10/67 (14.9%), 41-50 years 7/67 (10.4%) and 51-60 years 1/67 (1.4%). Whereas, those of female cases were distributed equally in 11-20 years and 21-30 years 9/33 (27.2% each) age groups, followed by 31-40 years age group 6/33 (18.2%), 41-50 years 5/33 (15.1%), more than 60 years 3/33 (9%) and 51-60 years 1/33 (3%). A total of 221 microorganisms were isolated from these patients on culturing various clinical samples like urine, pus, blood and endotracheal secretions. Out of 221, 198 (89.5%) were bacterial isolates and 23 (10.5%) were *Candida* spp. The majority of isolates were obtained from patients of 21-40 years 101/198 (51%) followed by those in  $\leq 20$  years 48/198 (24.2%). Maximum number of isolates were recovered from endotracheal secretions 126/198 (63.6%), followed by blood 33/198 (16.6%), urine 21/198 (10.6%) and pus 18/198 (9%) as shown in table 1.

**Table 1: Distribution of infections in different age groups in various clinical specimens**

Age groups (years)	Blood n(%)	Endotracheal secretions n(%)	Urine n (%)	Pus n(%)	Total no of isolates n(%)
≤20	8 (24.2%)	34 (26.9%)	3 (14.2%)	3(16.6%)	48 (24.2%)
21-40	17 (51.5%)	62 (49.2%)	10(47.6%)	12(66.6%)	101(51%)
41-60	3 (9%)	17 (13.4%)	3 (14.2%)	2 (11.1%)	25(12.6%)
>60	5 (15.1%)	13 (10.3%)	5 (23.8%)	1 (5.5%)	24(12.1%)
Total	33(16.6%)	126(63.6%)	21(10.6%)	18(9%)	198

Out of 198 bacterial isolates, 184 (92.9%) were gram-negative bacterial pathogens and 14 (7%) were gram-positive bacterial pathogens. Among 184 gram-negative bacterial isolates, most frequently isolated organism was *P. aeruginosa* 48/184 (26%). The second most common organism was *Acinetobacter* spp. 42/184 (22.8%), followed by *Enterobacter* spp. 28/184

(15.2%), *Klebsiella* spp. 27/184 (14.6%), *E. coli* 24/184 (13%), *Citrobacter* spp. 14/184 (7.6%) and *Proteus mirabilis* 1/184 (0.5%). Among a total of 14 gram-positive bacteria, 10 (71.4%) were *S. aureus* and 4/14 (28.6%) were coagulase negative staphylococci (CONS). All 23 *Candida* spp. was recovered from urine specimens.

**Table 2: Distribution of various clinical isolates among different clinical specimens**

Type of isolate	Endotracheal secretions n(%)	Blood n(%)	Urine n(%)	Pus n(%)	Total
<i>P. aeruginosa</i>	37(29.3%)	3(9%)	4(19%)	4(22.2%)	48(24.2)
<i>Acinetobacter</i> spp.	34(26.9%)	6(18%)	1(4.7%)	1(5.5%)	42(21.2)
<i>Enterobacter</i> spp.	19(15%)	6(18%)	1(4.7%)	2(11.1%)	28(14.1)
<i>Klebsiella</i> spp.	15(11.9%)	8(24.5%)	3(14.2%)	1(5.5%)	27(13.6)
<i>E. coli</i>	11(8.7%)	1(3%)	7(33.3%)	5(27.7%)	24(12.1)
<i>Citrobacter</i> spp.	6(4.7%)	8(24.5%)	—	—	14(7)
<i>S. aureus</i>	3(2.3%)	1(3%)	3(14.2%)	3(16.5%)	10(5)
CONS	1(0.7%)	—	1(4.7%)	2(11.1%)	4(2)
<i>P. mirabilis</i>	—	—	1(4.7%)	—	1(0.5)
Total	126	33	21	18	198

It was observed that out of 126 microbes isolated from endotracheal secretions, 102 (81%) were isolated singly called as monomicrobial episodes

(MME's), whereas, 24 (19%) microbes were present in combination with each other in ten polymicrobial episodes (PME's) as shown in table 3.

**Table 3: Distribution of microbes isolated from endotracheal secretions in 10 PME's and 102 MME's**

	MME's n(%)	PME's n(%)	Total n(%)
<i>P. aeruginosa</i>	29(28.4%)	8(33.3%)	37(29.3%)
<i>Acinetobacter</i> spp.	30(29.4%)	4(16.6%)	34(26.9%)
<i>Enterobacter</i> spp.	14(13.7%)	5(20.8%)	19(15%)
<i>Klebsiella</i> spp.	14(13.7%)	1(4.1%)	15(11.9%)
<i>E. coli</i>	7(6.8%)	4(16.6%)	11(8.7%)
<i>Citrobacter</i> spp.	5(4.9%)	1(4.1%)	6(4.7%)
<i>S. aureus</i>	2(1.8%)	1(4.1%)	3(2.3%)
CONS	1(0.9%)	—	1(0.7%)
Total	102(81%)	24(19%)	126(100%)

High level of antibiotic resistance was noticed for most of the antibiotics except imipenem and meropenem as shown in table 4 and figure 1.

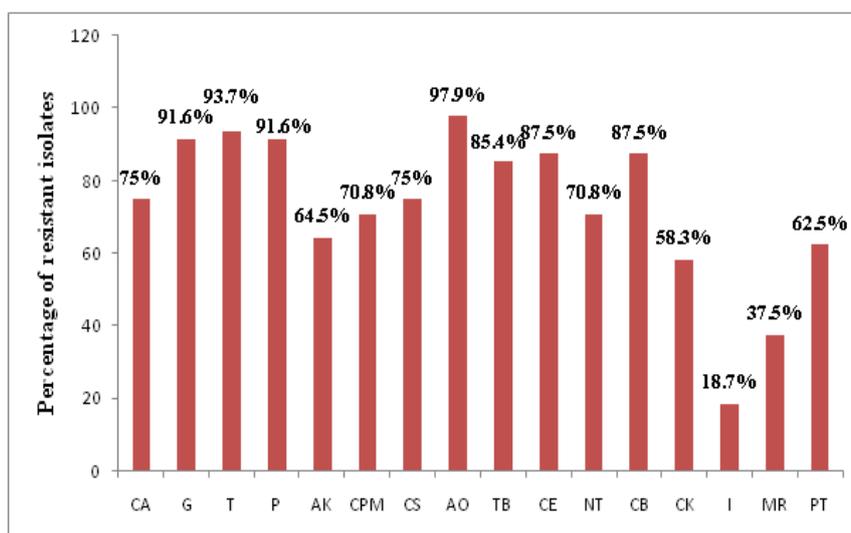
Out of 17 gram negative urinary isolates, 10 (58.8%) were resistant to norfoxacin and three (17.6%)

were resistant to nitrofurantoin. Both *S. aureus* and CONS were uniformly resistant to penicillin, norfloxacin, nitrofurantoin and cotrimoxazole whereas no resistance was observed against linezolid and vancomycin.

**Table 4: Antibiotic susceptibility pattern of gram-negative bacteria isolated from RICU patients**

	<i>E. coli</i>	<i>Acinetobacter</i> spp.	<i>Enterobacter</i> spp.	<i>Klebsiella</i> spp.	<i>Citrobacter</i> spp.	<i>P. mirabilis</i>	Total
	n=24	n=42	n=28	n=27	n=14	n=1	n=136
A	24(100)	42(100)	27(96.4)	27(100)	14(100)	1(100)	135(99.2)
G	24(100)	42(100)	26(92.8)	26(96.2)	9(64.2)	1(100)	128(94.1)
CU	21(87.5)	41(97.6)	25(89.2)	27(100)	14(100)	1(100)	129(94.8)
AK	12(50)	37(88)	20(71.4)	17(62.9)	11(78.5)	1(100)	98(72)
CE	21(87.5)	41(97.6)	25(89.2)	27(100)	12(85.7)	1(100)	127(93.3)
CF	23(95.8)	39(92.8)	25(89.2)	25(92.5)	10(71.4)	1(100)	123(90.4)
CK	12(50)	41(97.6)	13(46.4)	22(81.4)	11(78.5)	0	99(72.7)
I	3(12.5)	8(19)	3(10.7)	10(37)	5(35.7)	1(100)	30(22)
AO	24(100)	42(100)	27(96.4)	27(100)	14(100)	1(100)	135(99.2)
CA	19(79.1)	41(97.6)	25(89.2)	24(88.8)	13(92.8)	1(100)	123(90.4)
P	24(100)	42(100)	27(96.4)	27(100)	14(100)	1(100)	135(99.2)
CZ	22(91.6)	41(97.6)	24(85.7)	27(100)	14(100)	1(100)	129(94.8)
AC	23(95.8)	42(100)	27(96.4)	27(100)	13(92.8)	1(100)	133(97.7)
Cp m	22(91.6)	41(97.6)	23(82.1)	24(88.8)	14(100)	1(100)	125(91.9)
CO	24(100)	42(100)	26(92.8)	27(100)	14(100)	1(100)	134(98.5)
PT	20(83.3)	30(71.4)	21(75)	20(74)	7(50)	1(100)	99(72.7)
MR	6(25)	8(19)	12(42.8)	8(29.6)	3(21.4)	1(100)	38(27.9)

\*Figures in parenthesis indicate percentage of resistant strains. A= Ampicillin, G=Gentamicin, CU=Cefuroxime, AK=Amikacin, CE=Cefotaxime, CF=Ciprofloxacin, CK=Ceftizoxime, I=Imipenem, AO=Aztreonam, CA=Ceftazidime, P=Piperacillin, CZ=Cefazolin, AC= Amoxicillin clavulanic acid, CPM=Cefepime, CO=Cotrimoxazole, PT=Piperacillin tazobactam, MR=Meropenem.



**Figure 1: Antibiotic susceptibility pattern of *P. aeruginosa* isolated from various clinical specimens (Total no of isolates=48)**

CA=Ceftazidime, G=Gentamicin, T=Ticarcillin, P=Piperacillin, AK=Amikacin, CPM=Cefepime, CS= Cefoperazone, AO=Aztreonam, TB=Tobramycin, CE=Cefotaxime, NT=Netilmicin, CB=Carbenicillin, CK=Ceftizoxime, I=Imipenem, MR=Meropenem, PT=Piperacillin tazobactam.

**Table 5: Antibiotic susceptibility pattern of gram-positive bacteria isolated from various clinical samples. (*S. aureus*=10, CONS=4)**

	Tetracycline	Erythromycin	Cefazolin	Cefuroxime	Clindamycin	Oxacillin	Ciprofloxacin	Amoxyclav
<i>S.aureus</i>	7(70)	7(70)	8(80)	7(70)	3(30)	10(100)	9(90)	8(80)
CONS	1(25)	1(25)	1(25)	1(25)	1(25)	3(75)	1(25)	1(25)

\*Figures in parenthesis indicate percentage of resistant strains

Out of 198 bacterial isolates, two isolates were resistant to all the drugs tested, whereas three isolates were sensitive to all the drugs tested. 195 (98.4%) isolates showed resistant to three or more than three

drugs. A total of 12 microbes were isolated from different environmental samples taken at periodic intervals shown in table 6.

**Table 6: Distribution of various microorganisms isolated from environmental samples**

Site (n=30)	Number of bacterial isolates	Bacteria isolated
Floor and walls (4)	1	<i>S. aureus</i>
Beds (8)	5	<i>Acinetobacter</i> spp. (2) <i>E. coli</i> (1) <i>Enterobacter</i> spp. (1) <i>S. aureus</i> (1)
Ventilators (3)	1	<i>Enterobacter</i> spp. (1)
I/V stands (2)	—	—
Boyle's apparatus (2)	—	—
Normal saline (2)	1	<i>S. aureus</i> (1)
Wash basins (2)	1	<i>Enterobacter</i> spp. (1)
Suction machine (1)	1	<i>E. coli</i> (1)
Air plates (6)	2	<i>E. coli</i> (1) <i>Micrococcus</i> spp. (1)

## DISCUSSION

In the present study, during the study period of two years, 184 patients were admitted in the RICU. Hundred patients, in whom microbiological evidence of nosocomial infections noted were enrolled in the study, suggesting an infection rate of 54.4%. Most frequently reported NIs were ventilator associated pneumonias (63.6%) followed by blood stream infections (16.6%), urinary tract infections (10.6%), and surgical site infections (9%). During literature search variations in rate of ICU infections have been found from different studies such as rate of VAP was found to be 32% to 69%, blood stream infections were 16% to 28%, urinary tract infections were 13% to 30% and surgical site infections were 5 to 21% [7-11]. Variation in infection rates may be due to differences in surveillance methods, lack of uniformity in diagnostic criteria, lack of adequate systems to compare severity of illness and the difference in patient population.

In the current study, 87% infected patients were <60 years and only 13% were more than 60 years. Majority of the isolates (51%) were recovered from patients belonging to 21-40 years age group. 67% infected patients were male and 33% were female. These results are in accordance with Tennant and colleagues who reported 75% patients having infections were <70 years and 25% were >70 years, 56.4% infected patients were males and 43.6% were females [9].

More than eighty percent infections in ICU were due to gram-negative bacteria. Our results are concurrent with various other studies from different regions of the world. However, the distribution of microorganisms varies in different studies [12-16]. In this study, the predominant organism isolated was *P.*

*aeruginosa* (24.2%), followed by *A. baumannii* (21.2%), *Enterobacter* spp. (14.1%), *Klebsiella* spp. (13.6%), *E. coli* (12.1%), *Citrobacter* spp. (7%) and *S. aureus* (5%). Our results are in concurrent with study from Indonesia and Turkey in which *P. aeruginosa* was found to be the most common organism with isolation rates varying from nearly 20% to 26% [12, 13]. However, study from Ahmedabad have reported *E. coli* (25%) as the most frequent organism isolated followed by *Acinetobacter* spp. (15.6%) [14]. Another study from a Turkey hospital have found *S. aureus* (30.9%) and *Acinetobacter* spp. (26.8%) as the commonest pathogen [15]. Our study reports high level of antibiotic resistance to almost all the drugs. The gram-negative bacterial isolates such as *E. coli*, *Enterobacter*, *Klebsiella*, *Citrobacter* spp. showed high level of resistance to betalactam antibiotics such as ampicillin (90-100%), cefuroxime (87-100%), cefotaxime (87-100%), cefepime (82-100%), ceftazidime (79-100%), cefazolin (85-100%) as well as betalactam-betalactamase inhibitors i.e. amoxicillin/clavulanate (90-100%). Resistance to gentamicin (90-100%), ciprofloxacin (90-100%), aztreonam (90-100%) and cotrimoxazole (90-100%) were also found to be very high. However, most of these bacteria were highly sensitive to imipenem and meropenem with only 12-36% of the strains being resistant to imipenem and 19-42% of the strains being resistant to meropenem. *P. aeruginosa* strains were also showed high level of resistance to aztreonam (97.9%), piperacillin (91.6%), gentamicin (91.6%), carbenicillin (87.5%), cefotaxime (87.5%) and tobramycin (85.4%). Resistance to ceftazidime (75%), cefoperazone (75%), cefepime (70.8%), netilmicin (70.8%) were also high whereas resistance for ceftizoxime (58.3%), piperacillin/tazobactam (62.5%) and amikacin (64.5%) were slightly less. Resistance to imipenem and

meropenem was 18.7% and 37.5% respectively. So, it was observed that imipenem and meropenem were highly effective against *P. aeruginosa*. Meropenem and piperacillin/tazobactam was found to be 100% effective against all *P. aeruginosa* isolates obtained from blood whereas imipenem, amikacin and ceftizoxime were 100% effective against all *P. aeruginosa* isolates obtained from pus. *A. baumannii* showed high resistance (95-100%) to most of the drugs, however, imipenem and meropenem were also most effective drugs against this bacterium. *S. aureus* strains showed high resistance against tetracycline (70%), erythromycin (70%), ceftazolin (80%), cefuroxime (70%), and amoxicillin/clavulanate (80%). No resistance to linezolid and vancomycin was found in *S. aureus* as well as in CONS. All strains of *S. aureus* and 75% strains of CONS were methicillin resistant (MRSA). This scenario reflects extensive use of antibiotics in our ICU settings. \\

Survey from the 12 ICUs of the seven Indian cities showed that 71.4% of *Enterobacteriaceae* were resistant to ceftriaxone and 26.1% to piperacillin-tazobactam; 28.6% of the *P. aeruginosa* strains were resistant to ciprofloxacin, 64.9% to ceftazidime and 42.0% to imipenem, 87.5% of *S. aureus* strains were methicillin resistant strains [17]. Another study from India on ICU patients also reported that the antibiotics that remained most active against all gram-negative organisms for two years were only imipenem, piperacillin-tazobactam and amikacin [18]. In a study from Iran, amikacin and imipenem were found to be most active antibiotics against gram-negative microorganisms (54% and 46% respectively) [16]. A study from Turkey also showed that 82-95% isolates of *S. aureus* and 98.6% strains of CONS were methicillin resistant and all were resistant to ampicillin and tetracycline and in the same study 71.3-98.1% resistance to third generation cephalosporins were noticed in *P. aeruginosa* [13].

## CONCLUSION

Surveillance of the microbial etiology of nosocomial infections over prolonged time periods not only can provide important information for day to day decision making in antimicrobial therapy but also reflects local trends and shifts in etiology and drug resistance. The high rate of antibiotic resistance in the present study shows that imipenem and meropenem are the only reliable agents for the empirical treatment of ICU infections. However, the current scenario appears to be the result of ineffective infection control measures and antibiotic policies. Hence, for proper management of critically ill patients in ICUs, hospital antibiotic policies need frequent revisions. Otherwise very few options will be left in future in the antibiotic armamentarium to control the medical disaster with strains virtually untreatable with current spectrum of antimicrobials.

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