



## Lower Respiratory Tract Infections in Intensive Care Units. A Four Year Study from North India

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### Authors' contributions

This work was carried out in collaboration between all authors. Author NB designed the study, carried out the experimental process and wrote the draft of the manuscript. Author DK managed the literature searches. Authors AF, HB and SL helped in analyses of the results. Author PK helped in the concept, literature research, analysis and compilation of the final draft. All authors read and approved the final manuscript.

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### ABSTRACT

**Title:** Lower respiratory tract infections in intensive care units. A four year study from North India.  
**Study Design:** Prospective study  
**Place and Duration of Study:** Sher-i-Kashmir Institute of Medical Sciences, Srinagar Kashmir. Four years (July 2010 and June 2014).  
**Methodology:** A prospective analysis of respiratory specimens from various intensive care units (ICUs) was done over a period of four years. Antimicrobial susceptibility of culture positive isolates to various antibiotics was performed as per Clinical Laboratory Standards Institute (CLSI) guidelines.

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Gram-negative bacteria (GNB) were screened for extended spectrum  $\beta$ -lactamase (ESBL) and metallo- $\beta$ -lactamase (MBL) production; whereas methicillin and vancomycin resistance was searched in *staphylococci* and *enterococci* isolates respectively.

**Results:** The frequencies of Gram-positive and Gram-negative bacteria were 26% and 68% respectively with yeast recovered in 8% of the specimens. *K. pneumoniae* and *Acinetobacter* spp were the most common Gram-negative bacteria and *S. aureus* the most common Gram-positive one. High level resistance to all the antimicrobials was seen; with *K. pneumoniae* being the most multidrug resistant GNB isolated in the ICU setting. ESBL production was also highest in *K. pneumoniae* isolates (67.1%). Also 59.6% of *Acinetobacter* isolates were found to be MBL producers. Methicillin resistance was seen in 48% of *S. aureus* and 85.5% of coagulase negative staphylococci (CoNS) isolates with vancomycin resistance seen in 6.7% of enterococcal isolates.

**Conclusion:** An increasing trend over the years in the antibiotic resistance of respiratory pathogens in ICUs of this north Indian state was seen that calls for urgent measures to limit their continued rise.

**Keywords:** ESBL; gram negative bacteria; MBL.

## 1. INTRODUCTION

Lower respiratory tract infections are some of the most common bacterial infections among patients admitted in intensive care units (ICUs) being associated with high mortality, ranging from 22% to 71% [1]. Due to frequent empirical use of antibiotics in the sick patients housed there, ICUs are faced with rapid emergence and spread of multidrug resistant bacteria [2,3]. Additionally, the frequent need for tubes and lines like endotracheal tubes, central and peripheral vascular access lines, urinary catheters etc, for prolonged periods places these patients at a high risk of nosocomial infections due to antimicrobial resistant bacteria like vancomycin resistant *Enterococci*, methicillin resistant *S. aureus*, extended spectrum  $\beta$ -lactamase (ESBL) and metallo- $\beta$ -lactamase (MBL) producing Gram-negative bacteria etc [4].

According to similar studies in Europe and USA, more than 20% of patients admitted to European intensive care units (ICUs) develop an ICU acquired infection. A high prevalence of decreased antibiotic susceptibility among Gram-negative bacilli has been reported from ICU patients in France, Belgium, and Germany, during 1990 and 1991, the United States between 1990 and the 1993, and Belgium and Sweden during 1994 and 1995 [5]. The emergence of MDR bacteria is an increasing cause of nosocomial infections in ICUs and is associated not only with increased morbidity and mortality, but also with increased treatment costs as a result of frequent empirical antibiotic treatment failure and lengthy hospital stay [6].

Drug resistance has rendered difficult the antimicrobial therapy in India like everywhere

else [7,8] and highly resistant bacteria like the MBL producing Gram-negative bacteria are a common occurrence in the hospital settings especially the intensive care units. Recently the New Delhi metallo  $\beta$ -lactamase (NDM) producing multidrug resistant Gram-negative bacteria have been reported from Kashmir [9]. These have serious implications for the management of critically ill patients in ICUs, limiting the utility of beta-lactam antibiotics, fluoroquinolones and aminoglycosides. However, no systematic study regarding the pattern of resistance among the pathogens recovered from the respiratory specimens in ICU patients is available and since empirical drug therapy is instituted in these patients way before culture reports and results of antimicrobial testing become available, an understanding of the local ecology of bacteria and their susceptibility profiles can serve as an important guide to the choice of the antimicrobials.

The present study was designed to identify the microbiological profile and susceptibility pattern of the organisms isolated from lower respiratory tract of patients admitted in the ICUs of our hospital which is the only tertiary care institute in the north Indian state of Jammu and Kashmir.

## 2. MATERIALS AND METHODS

A prospective analysis, of the isolates recovered from lower respiratory tract (LRT) specimens of patients admitted to the ICUs at Sher-i-Kashmir Institute of Medical Sciences (SKIMS) was carried out between July 2010 and June 2014. The intensive care units included the surgical ICU (13-bed ICU), neonatal ICU (8-bed ICU), medical ICU (8-bed ICU) and cardiac surgical ICU (6-bed ICU). Sputum samples, tracheal

aspirates, bronchial lavages and endotracheal tube tips received in the Department of Microbiology at SKIMS were processed for the recovery of bacterial pathogens according to standard microbiological procedures [10]. Gram staining of the samples was done initially to guide the clinicians in deciding the appropriate treatment option. Samples were plated onto blood agar, MacConkey agar and chocolate agar plates and incubation at 37°C for 18-24 hours. Endotracheal tube tips were placed in Robertson's cooked meat broth (RCM) and subcultured the next day in case the primary culture plate was sterile and the RCM broth showed turbidity. Single or mixed growth (two predominant colonies) isolated from samples inoculated onto media plates were observed for colony characteristics. Relevant spot tests (catalase, coagulase and oxidase) and biochemical tests were performed to identify the organisms up to species level.

Antimicrobial susceptibility testing was done on Muller Hinton agar plates by Kirby-Bauer disc diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines [11]. Isolates collected from the same specimen source of the same patient were excluded. The susceptibility patterns of the bacterial pathogens were determined following the panel of antimicrobial agents as recommended by CLSI 2010 with zone diameters measured in millimeters [11]. Gram-negative clinical isolates were screened for ESBL (using ceftazidime and ceftazidime plus clavulanic acid discs), and MBL production (done with combined disc test using imipenem and imipenem plus EDTA); whereas methicillin and vancomycin resistance was searched for in *S. aureus* and *Enterococci* isolates respectively as per CLSI guidelines.

Multidrug resistance (MDR) for Gram-negative organisms was defined as resistance to three or more classes of antimicrobial agents, while pandrug resistant strains exhibited resistance to all classes [12]. The MDR strains of *Mycobacterium tuberculosis* were not addressed in this study.

*Staphylococcus aureus* ATCC 25923 and *E. coli* ATCC 35218 were used as a quality control strains for disc diffusion method. Culture media, antibiotic discs and reference strains used in the study were procured from HiMedia Pvt Laboratories, Mumbai. Ethical clearance was sought from the institute's ethical committee.

### 3. RESULTS

A total of 2310 respiratory specimens were received during the study period out of which 1740 (75.3%) were culture positive (bacterial as well as fungal); 311 (13.5%) were sterile and 259 (11.2%) grew organisms generally regarded as contaminants. The yield of the organisms was higher in the tracheal/bronchial lavage samples (n=695, 40%) as compared to sputum samples (n=598, 34.4%) and endotracheal tube tip specimens (n=447, 26%).

Patients enrolled in the study included 1623 (70.3%) males and 687 (29.7%) females. Most of the culture positive samples were received from the surgical ICU (n= 1225, 70.4%) followed by the medical ICU (n=291, 16.7%), the neonatal ICU (n=182, 10.5%) and the cardiac surgical ICU (n=42, 2.4%). Most (n=627, 36.0%) of the culture positive isolates were from patients in the age group of 60-69 yrs. Majority of the patients had underlying respiratory (43.7%) or neurological disorders (16.7%).

Out of the 1740 positive cultures, bacterial isolates were recovered from 1601 (92%) and fungal isolates from 139 (8%) cultures. The most common organisms isolated are shown in Table 1. The frequencies of Gram-positive and Gram-negative bacteria were 26% (n=453) and 68% (n=1148) respectively with yeast recovered in only 8% of the specimens. *Klebsiella pneumoniae*, *Acinetobacter* spp and *Pseudomonas aeruginosa* were the most common isolates among the Gram negative organisms whereas *Staphylococcus aureus* and *Enterococcus* spp comprised the majority of the Gram positive ones. Of the 237 *S. aureus* isolates 114 (48%) were methicillin resistant (MRSA). Among the 62 coagulase-negative staphylococcal isolates 53 (85.5%) were methicillin resistant (MRSS). Vancomycin resistance was seen in 9 (6.7%) of the enterococcal isolates, with all them being *E. faecium*.

ESBL producing *K. pneumoniae* accounted for 67.1% of the total number of *Klebsiella* strains isolated. Likewise, 47.4% of *E. coli*, 45.6% of *Enterobacter* spp and 34.6% of *Citrobacter* spp. were ESBL producers, whereas none of the *Proteus* spp and *Morganella morganii* were positive for the enzyme. Also 59.6% of *Acinetobacter* and 47.3% of *P. aeruginosa* isolates were found to be MBL producers (Table 2). An increasing trend in the prevalence of these

enzymes (ESBL, MBL) in the isolates was seen over the years.

Most frequently isolated mixed bacterial cultures included a combination of *K. pneumoniae* and *P. aeruginosa* (n=14, 41.2%) followed by *K. pneumoniae* and *E. coli* (n=10, 29.4%), *P. aeruginosa* and *S. aureus* (n=7, 20.6%) *P. aeruginosa* and *P. mirabilis* (n=2, 5.8%) and *A. baumannii* and *K. pneumoniae* (n=1, 2.9%).

**Table 1. Most common organisms recovered from the respiratory samples**

Organism	Total (N=1740)
<b>Gram positive organisms</b>	N (%)
<i>S. aureus</i>	230 (13.2)
<i>S. pneumoniae</i>	27 (1.5)
<i>Enterococcus</i> spp	134 (7.7)
CoNS	62 (3.6)
Total	453 (26.0)
<b>Gram negative organisms</b>	
<i>E. coli</i>	66 (3.8)
<i>K. pneumoniae</i>	482 (27.7)
<i>Enterobacter cloacae</i>	57 (3.3)
<i>Citrobacter</i> spp	52 (2.9)
<i>Proteus</i> spp	23 (1.3)
<i>P.aeruginosa</i>	184 (10.6)
<i>Acinetobacter</i> spp	239 (13.7)
<i>Morganella morganii</i>	11 (0.6)
<i>Mixed bacterial etiology</i>	34 (1.9)
Total	1148 (66)
<b>Yeast</b>	
<i>C. albicans</i>	72 (4.1)
<i>C. glabrata</i>	41 (2.4)
<i>C. krusei</i>	19 (1.1)
<i>C. parapsilosis</i>	7 (0.4)
Total	139 (8)

The yearly antibiogram of organisms belonging to the family *Enterobacteriaceae* is given in Tables 3 and 4, that of the non-fermenting bacteria is depicted in Table 5 and Gram-positive bacteria in Table 6.

**Table 2. Year-wise prevalence of ESBL and MBL producing Gram-negative bacteria**

Organism	2010 N (%)	2011 N (%)	2012 N (%)	2013 N (%)	Total N (%)
<b>ESBL</b>					
<i>K. pneumoniae</i>	56 (45.5)	80 (60.6)	86 (71.1)	118 (90.1)	340 (67.1)
<i>Escherichia coli</i>	5 (26.3)	4 (33.3)	12 (57.1)	15 (62.5)	36 (47.4)
<i>Enterobacter</i> spp	2 (14.3)	5 (31.3)	7 (58.3)	12 (80)	26 (45.6)
<i>Citrobacter</i> spp	3 (33.3)	0	8 (61.5)	7 (43.7)	18 (34.6)
<b>MBL</b>					
<i>Acinetobacter</i> spp	19 (48.7)	37 (71.2)	32 (47.1)	55 (67.9)	143 (59.6)
<i>P. aeruginosa</i>	11 (35.5)	17 (39.5)	29 (47.5)	41 (56.9)	98 (47.3)

ESBL: Extended spectrum beta lactamase; MBL: metallo-beta lactamase

#### 4. DISCUSSION

Our data provides an understanding of the antibiotic resistance patterns of commonly isolated organisms in ICU patients in north India. The scene is alarming and clearly demonstrates that drug resistance is on the rise and clinicians are left with very few options for treating patients with serious respiratory infections in the ICU settings that constitute the second most common cause of hospital acquired infections [13].

Rise in the antimicrobial resistance among respiratory pathogens in ICU's due to inadvertent and non judicious administration of antibiotics generally before the availability of the culture results, is a matter of potential concern worldwide. A prospective point prevalence study conducted in 1265 ICU's in 75 countries demonstrated that patients who had longer ICU stays had higher rates of infection, especially due to resistant *Staphylococci*, *Acinetobacter*, *Pseudomonas* and *Candida* species. Moreover, the mortality of infected patients in ICU was more than twice that of non-infected patients [14].

We saw an overall preponderance of Gram-negative (66%) bacteria in the LRT infections, with *K. pneumoniae* being the predominant organism followed by *Acinetobacter* and *Pseudomonas*. This is not surprising since the former (GNB) are implicated frequently in causing disease and develop resistance more rapidly and extensively than the latter [15]. Moreover enterobacterial species rapidly colonize the oropharynx of many hospitalized patients regardless of whether they receive antimicrobials. Numerous studies have implicated these organisms in varying frequency to be the leading cause of LRTI's in ICU settings [2,13,16-18].

**Table 3. Antimicrobial resistance pattern of lactose fermenting *Enterobacteriaceae***

Years	<i>E. coli</i> (n=76)				<i>K. pneumoniae</i> (n=507)				<i>Enterobacter spp</i> (n=57)			
	2010	2011	2012	2013	2010	2011	2012	2013	2010	2011	2012	2013
<b>No of isolates</b>	<b>19</b>	<b>12</b>	<b>21</b>	<b>24</b>	<b>123</b>	<b>132</b>	<b>121</b>	<b>131</b>	<b>14</b>	<b>16</b>	<b>12</b>	<b>15</b>
Ceftriaxone	42.1	83.3	57.1	75	78.9	78.8	81.8	87.0	57.1	68.8	83.3	80
Ceftazidime	47.4	75	52.4	58.3	76.4	75	71.9	93.1	50	50	75	80
Cefipime	26.3	50	57.1	58.3	74.8	74.2	77.6	78.6	42.9	56.2	50	66.7
Ampicillin+sulbactam	73.7	58.3	85.7	83.3	-	-	-	-	64.3	62.5	100	93.3
Piperacillin+tazobactam	57.8	66.7	61.9	62.5	78.9	78.8	79.3	91.6	57.1	75	58.3	73.3
Ticarcillin+clavulanic acid	68.4	58.3	57.1	62.5	-	-	-	-	64.3	81.3	75	73.3
Co-trimoxazole	73.7	83.3	71.4	54.2	70.7	69.7	81	93.9	57.1	68.7	66.7	66.7
Amikacin	47.4	75	52.4	58.3	82.1	69.7	81	80.2	35.7	56.3	66.7	66.7
Gentamicin	52.6	75	61.9	58.3	78.1	74.2	76.9	86.3	50	50	58.3	60
Quinolones	63.2	83.3	76.2	87.5	77.2	75	90.1	88.6	64.3	93.8	83.3	86.7
Imipenem	36.8	50	71.4	75	63.4	65.9	76.0	92.4	50	62.5	66.7	60
Polymyxin-B	0	0	0	0	0	0	0	0	0	0	0	0
Tetracycline	47.4	58.3	76.2	70.8	71.5	71.2	76.0	94.0	78.6	62.5	75	66.7

Numbers depict percentages of resistant isolates

**Table 4. Antimicrobial resistance pattern of non-lactose fermenting *Enterobacteriaceae***

Year	<i>Citrobacter spp</i> (n=52)				<i>Proteus spp</i> (n=25)				<i>Morganella spp</i> (n=11)			
	2010	2011	2012	2013	2010	2011	2012	2013	2010	2011	2012	2013
<b>No of isolates</b>	<b>9</b>	<b>13</b>	<b>14</b>	<b>16</b>	<b>7</b>	<b>4</b>	<b>8</b>	<b>6</b>	<b>1</b>	<b>5</b>	<b>2</b>	<b>3</b>
Ceftriaxone	55.6	69.2	71.4	87.5	57.1	100	62.5	66.7	100	80	0	66.7
Ceftazidime	66.7	61.5	64.3	87.5	57.1	75	37.5	66.7	0	80	50	66.7
Cefipime	44.4	53.9	64.3	85.7	28.6	25	62.5	66.7	0	40	50	100
Ampicillin+sulbactam	33.3	69.2	92.9	100	42.9	50	100	83.3	100	60	50	66.7
Piperacillin+tazobactam	44.4	61.5	64.3	85.7	71.4	50	75	66.7	0	40	50	66.7
Ticarcillin+clavulanic acid	66.7	61.5	64.3	93.8	57.1	50	62.5	100	100	60	0	100
Co-trimoxazole	66.7	53.9	64.3	75	42.9	0	50	83.3	0	60	50	66.7
Amikacin	55.6	61.5	64.3	62.5	42.9	25	62.5	50	100	60	100	66.7
Gentamicin	66.7	61.5	57.2	62.5	42.9	25	62.5	50	100	60	100	66.7
Quinolones	77.8	61.5	78.6	81.3	57.1	75	75	100	0	60	100	100
Imipenem	44.4	46.2	64.3	81.3	57.1	50	62.5	83.3	0	40	100	100
Polymyxin-B	0	0	0	0	100	100	100	100	100	100	100	100
Tetracycline	66.7	61.5	57.2	56.3	57.1	25	62.5	66.7	100	40	50	66.7

**Table 5. Antimicrobial resistance pattern of non-fermenting bacteria**

Years	<i>Acinetobacter spp.</i> (n=240)				<i>Pseudomonas spp.</i> (n=207)			
	2010	2011	2012	2013	2010	2011	2012	2013
<b>No of isolates</b>	<b>39</b>	<b>52</b>	<b>68</b>	<b>81</b>	<b>31</b>	<b>43</b>	<b>61</b>	<b>72</b>
Ceftriaxone	64.1	75	72.1	93.8	-	-	-	-
Ceftazidime	59	71.2	75	80.3	51.6	62.8	63.9	68.1
Cefipime	48.7	67.3	82.4	77.8	-	-	-	-
Ampicillin+sulbactam	64.1	65.4	80.9	90.1	-	-	-	-
Piperacillin+tazobactam	53.8	63.5	70.6	74.1	38.7	55.8	57.4	65.3
Ticarcillin+clavulanic acid	59	75	75	79.0	45.2	62.8	67.2	77.8
Co-trimoxazole	46.2	61.5	72.1	74.1	-	-	-	-
Amikacin	46.2	55.8	61.8	76.5	41.9	53.5	54.1	73.6
Gentamicin	43.6	59.6	63.2	77.8	48.4	48.8	55.7	77.8
Quinolones	66.7	78.9	73.5	77.8	61.3	53.5	65.6	69.4
Imipenem	53.8	51.9	64.7	81.5	35.5	46.5	55.7	66.7
Polymyxin-B	0	0	0	0	0	0	0	0
Tetracycline	61.5	75	72.1	65.4	-	-	-	-

In the ICU of a tertiary care hospital in South India, *K. pneumoniae* and *P. aeruginosa* were the commonest isolated organisms [7]. The rise of resistant Gram negative organism was also observed in a 2-year study from Western Indian setting where the trend was in favor of increasingly resistant *Enterobacteriaceae* [8]. A study conducted in 12 ICU's in seven Indian cities showed *Enterobacteriaceae* (46%), *Pseudomonas* (27%), *Acinetobacter* spp. (6%), *Candida* spp. (8%), *S. aureus* (6%) as causative agents of nosocomial infections [19].

ESBL production was seen in 420 (57.7%) isolates of the various species of *Enterobacteriaceae* recovered, with *K. pneumoniae* being the predominant organism positive for this enzyme (67.1%). Likewise a higher percentage of *Acinetobacter* isolates (59.6%) were found to be positive for the presence of MBL enzyme. Emergence of ESBL producing organism has become a burgeoning problem across all ICUs across the country. A recent study from north India demonstrated that among 78% ESBL producers of the 250 Gram-negative organisms, extensive drug resistance was seen in 45%, followed by multidrug resistance seen in 27%. Also 6% of the isolates were pan drug resistant. In this study, among the extensive-drug resistant organisms, seven (6.1%) organisms were New Delhi metallo- $\beta$ -lactamase-1 (NDM-1) producers and five (4.4%) organisms were NDM-2 producers [20]. NDM production has been reported from our center too very recently. This indicates a high level of resistance creeping in that limits the options in the management of patients. High level resistance to all the antimicrobials tested

especially beta-lactam antibiotics, cephalosporins and quinolones was seen in this study; with *K. pneumoniae* being the most multidrug resistant Gram negative organism isolated in the ICU setting.

Amongst the Gram positive bacteria, *S. aureus* was the most common organism recovered with 48% isolates being resistant to methicillin (MRSA). On the other hand 85.5% of CoNS were found exhibit methicillin resistance. *Enterococcus* spp. was the second most frequent GPC isolated, with 6.7% of them exhibiting resistance to vancomycin (VRE). Till very recently the antimicrobial resistance scenario in India was quite different from the Western settings where the major share of hospital associated infections since the 1980's are caused by Gram positive cocci like *S. aureus* and *Enterococci* [21]. However, the scene in most ICUs is changing and emergence of methicillin resistant organisms and the ones exhibiting glycopeptide resistance is on the rise. The emergence of VRE represents a worst case scenario as nosocomial spread of these pathogens especially in areas like the ICU's may create a reservoir of mobile resistance genes for other more virulent organisms like *S. aureus* [22].

Although variable sensitivity was seen to other antimicrobial agents like clindamycin and co-trimoxazole; none of the Gram-positive organisms exhibited resistance to linezolid. High resistance in Gram-positive cocci to routinely used antibiotics against these organisms is a distinct worry that seems to be prevalent across the country [23-25] limiting the options available for management of such cases.

**Table 6. Antimicrobial resistance pattern of gram positive bacteria**

Year	<i>S. aureus</i> (n=237)				CoNS (n=62)				<i>S. pneumoniae</i> (n=27)				Enterococcus (n=134)			
	2010	2011	2012	2013	2010	2011	2012	2013	2010	2011	2012	2013	2010	2011	2012	2013
<b>No of isolates</b>	<b>47</b>	<b>66</b>	<b>73</b>	<b>51</b>	<b>13</b>	<b>19</b>	<b>14</b>	<b>16</b>	<b>6</b>	<b>8</b>	<b>5</b>	<b>8</b>	<b>27</b>	<b>28</b>	<b>36</b>	<b>43</b>
Penicillin	89.4	92.4	96	100	84.6	84.2	100	100	0	0	0	0	92.6	96.4	91.7	100
Ampicillin	-	-	-	-	-	-	-	-	-	-	-	-	52	57.1	64	88.4
Cefoxitin	42.6	42.4	53.4	52.9	38.5	42.1	42.9	56.3	-	-	-	-	-	-	-	-
Clindamycin	72.3	74.2	71.2	76.5	61.5	73.7	71.4	81.3	33.3	25	20	37.5	-	-	-	-
Cotrimoxazole	78.7	77.3	76.7	74.5	76.9	84.2	85.7	81.3	16.7	37.5	0	50	-	-	-	-
Vancomycin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.6	16.3
Linezolid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Quinolones	80.8	80.3	89.0	80.4	84.6	94.7	85.7	93.7	0	50	66.7	100	70.4	64.3	86.1	90.7
Erythromycin	89.4	92.4	82.2	74.5	100	100	92.9	68.7	50	12.5	40	37.5	-	-	-	-
Chloramphenicol	-	-	-	-	-	-	-	-	0	0	80	25	-	-	-	-

## 5. CONCLUSION

Our data thus is indicative of a worrisome rise in the antimicrobial resistance of respiratory pathogens in ICUs of this north Indian state that calls for urgent measures to limit their continued rise. These measures among others include proper selection of antibiotics, infection control practices, antibiotic stewardship, proper de-escalation and regular surveillance to assess the local ecology so as to guide proper antibiotic therapy.

## CONSENT

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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