

Burden of extensively drug-resistant and pandrug-resistant Gram-negative bacteria at a tertiary-care centre

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Abstract

The emergence of resistance to multiple antimicrobial agents in Gram-negative bacteria is a significant threat to public health, as it restricts the armamentarium of the clinician against these infections. The aim of this study was to determine the burden of extensively drug-resistant (XDR) and pandrug-resistant (PDR) Gram-negative bacteria at a tertiary-care centre. Antimicrobial susceptibility testing of 1240 clinical isolates of Gram-negative bacteria obtained from various clinical samples during the study period was carried out by the Kirby-Bauer disc diffusion method. Minimum inhibitory concentration of all antibiotics including tigecycline and colistin was determined by Vitek-2 automated susceptibility testing system. Out of 1240 isolates of Gram-negative bacteria, 112 isolates (9%) were resistant to all the antibiotics tested by Kirby-Bauer disc diffusion method. This finding was corroborated by Vitek-2. In addition, Vitek-2 found that 67 isolates were resistant to all antibiotics except tigecycline and colistin. A total of 30 isolates were susceptible to only colistin, and four isolates were susceptible to only tigecycline. It was also found that six isolates (excluding five isolates of *Proteus* spp.) were resistant to both colistin and tigecycline. Thus, 101 (8.1%) out of 1240 isolates were XDR and 11 isolates (0.9%) were PDR. The findings of this study reveal increased burden of XDR and PDR Gram-negative bacteria in our centre. It also highlights the widespread dissemination of these bacteria in the community. This situation warrants the regular surveillance of antimicrobial resistance of Gram-negative bacteria and implementation of an efficient infection control program.

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Keywords: Colistin, extensively drug-resistant (XDR), Gram-negative bacteria, pandrug-resistant (PDR), tigecycline, Vitek-2

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Introduction

Of late, the medical community worldwide has been witness to an increase in infections due to Gram-negative bacteria, which are resistant to many classes of antibiotics [1]. These infections are an important cause for prolonged hospitalization, leading to increased treatment costs and poor patient outcome in the form of increased morbidity and mortality [2]. These resistant pathogens were earlier considered to be primarily nosocomial pathogens, but it is now evident that they have spread to the community [3]. The emergence of resistance to multiple

antimicrobial agents in pathogenic bacteria has become a significant threat to public health, as there are fewer, or sometimes even no, effective antimicrobial agents available for infections caused by these bacteria [4].

In the medical jargon to date, there is no consensus on the definitions and use of terms such as 'multidrug resistant' (MDR), 'extensively drug resistant' (XDR) and 'pandrug resistant' (PDR), which depict resistance in multidrug-resistant organisms [4]. A proposition for defining these resistant bacteria was discussed in a joint program by European Centre for Disease Prevention and Control (ECDC) and the US Centers for Disease Control and Prevention (CDC). They formulated few definitions and defined XDR bacteria as 'isolates being non-susceptible to at least one agent in all but 2 or fewer antimicrobial categories listed in the Clinical and Laboratory Standards Institute (CLSI) guidelines' and PDR bacteria as 'isolates

being non-susceptible to all agents in all antimicrobial categories for each bacterium' [4].

This study was carried out with an aim to determine the burden of XDR and PDR Gram-negative bacteria at a tertiary-care centre in Pune, India.

Materials and methods

This study was carried out from July to September 2014 at a large tertiary-care centre. A total of 1240 nonrepetitive clinical isolates of Gram-negative bacteria were identified from various clinical specimens received in the microbiology laboratory with the help of conventional phenotypic methods.

Antimicrobial susceptibility testing was carried out by Kirby-Bauer disc diffusion method, and the results were interpreted according to CLSI guidelines [5].

Minimum inhibitory concentration (MIC) for all the isolates which were resistant to all the antibiotics by disc diffusion method was determined by Vitek-2 automated susceptibility testing method.

Results

A total of 1240 nonrepetitive clinical isolates of Gram-negative bacteria were identified by conventional phenotypic methods from various clinical samples received in the microbiology laboratory of a tertiary-care centre.

The most commonly isolated Gram-negative bacteria was *Escherichia coli* (625/1240), followed by *Klebsiella pneumoniae* (269/1240), *Pseudomonas aeruginosa* (172/1240), *Acinetobacter baumannii* (123/1240), *Proteus* spp. (40/1240), *Enterobacter* spp. (6/1240) and others (5/1240).

Out of these 1240 isolates of Gram-negative bacteria, 112 isolates (9%) were resistant to all the antibiotics tested by Kirby-Bauer disc diffusion method. The distribution of these resistant isolates is listed in Table 1. The most common isolate found to be resistant to all antibiotics tested was *P. aeruginosa*, followed by *K. pneumoniae*, *A. baumannii*, *E. coli* and *Proteus* spp.

The most common sample from which these 112 isolates were obtained was urine (37.5%), followed by wound swab/pus (22.3%), tracheal aspirates (17.9%), blood (7.1%), cerebrospinal fluid (7.1%), central line tip (4.5%) and other miscellaneous samples (3.6%). The sample-wise distribution of these resistant isolates is shown in Fig. 1.

The ward-wise distribution of isolates is shown in Fig. 2. Most of the resistant isolates were obtained from acute wards (42.9%) and intensive care units (ICUs) (29.5%), followed by other wards (23.2%) and the outpatient department (OPD) (4.4%).

TABLE 1. Species distribution of extensively resistant and pandrug-resistant Gram-negative bacteria by disc diffusion method (n = 112)

Sample	Organism	Total isolates	Resistant isolates	%
1.	<i>Escherichia coli</i>	625	18	2.9
2.	<i>Klebsiella pneumoniae</i>	269	32	11.9
3.	<i>Pseudomonas aeruginosa</i>	172	34	19.8
4.	<i>Acinetobacter baumannii</i>	123	19	15.4
5.	<i>Proteus</i> spp.	40	5	12.5
6.	<i>Enterobacter</i> spp.	6	2	33.3
7.	<i>Raoultella ornithinolytica</i>	1	1	100
8.	<i>Hafnia alvei</i>	1	1	100
9.	<i>Salmonella typhi</i>	1	—	—
10.	<i>Stenotrophomonas maltophilia</i>	1	—	—
11.	<i>Serratia marcescens</i>	1	—	—

MIC of all antibiotics was determined by Vitek-2 automated susceptibility testing system using GN-AST cards. It was found that 67 isolates were resistant to all antibiotics except tigecycline and colistin. A total of 30 isolates were susceptible to only colistin, of which 29 were *P. aeruginosa* and one was *A. baumannii*. Four isolates were susceptible to only tigecycline, out of which two were *K. pneumoniae* and two were *A. baumannii*. A total of six isolates were resistant to both colistin and tigecycline, out of which three were *P. aeruginosa*, two were *K. pneumoniae* and one was *Hafnia alvei*. As *Proteus* spp. are intrinsically resistant to tigecycline and colistin, testing for these antibiotics against *Proteus* spp. was not done by Vitek-2. Thus, 101 (8.1%) of 1240 isolates were XDR, and 11 isolates (0.9%) were PDR. The species distribution of XDR and PDR Gram-negative bacteria is shown in Table 2.

The most common sample from which PDR isolates were obtained was urine (6/11), followed by tracheal aspirate (3/11) and pus (2/11). These PDR isolates were mainly obtained from ICUs (6/11) and acute wards (5/11). No PDR isolate was obtained from other wards or OPD.

Discussion

Antimicrobial resistance is a worldwide problem that knows no international boundaries and can spread between continents [6]. Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant threat to public health, as there are fewer, or sometimes even no, effective antimicrobial agents available for infections caused by these bacteria [4]. Of late, terms such as 'multidrug resistance' have been used in medical literature to describe isolates of *Mycobacterium tuberculosis* resistant to rifampicin and isoniazid. A group of international experts came together in a joint initiative of the ECDC and CDC to deliberate and describe different patterns of resistance found in healthcare-associated,

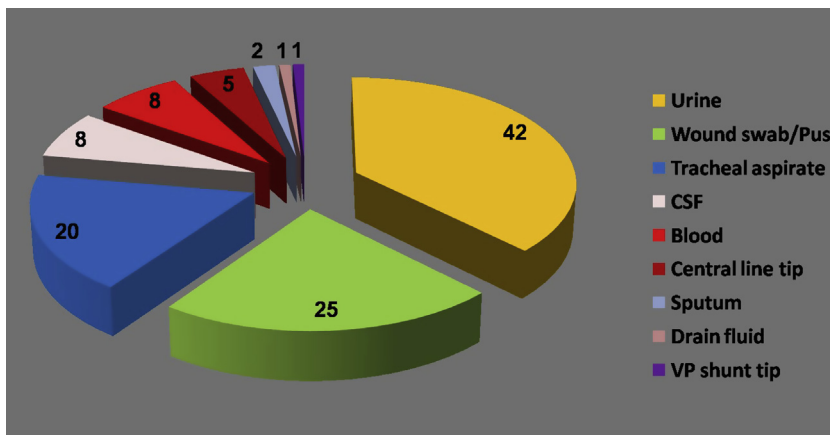


FIG. 1. Sample-wise distribution of resistant isolates.

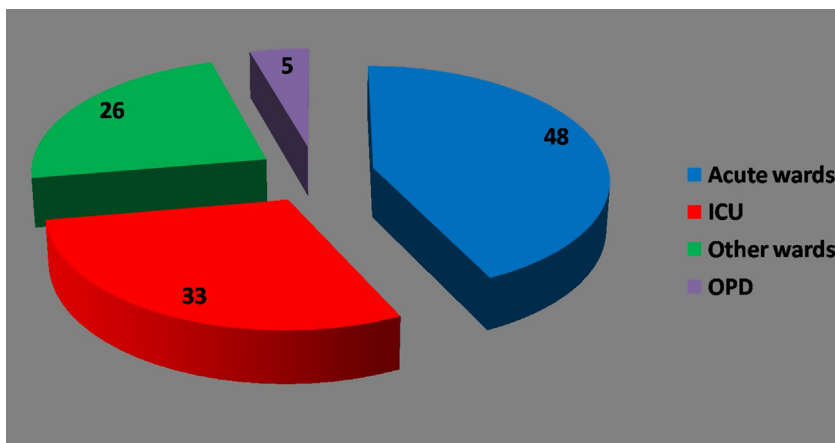


FIG. 2. Ward-wise distribution of resistant isolates.

antimicrobial-resistant bacteria. According to ECDC and CDC, MDR is defined as nonsusceptibility to at least one agent in three or more antimicrobial categories, XDR is defined as the nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories while PDR is defined as nonsusceptibility to all agents in all antimicrobial categories for each bacterium [4,7].

During the last few years, there has been a significant increase in infections caused by Gram-negative bacteria, which are resistant to several classes of antibiotics [1]. These infections are an important cause for prolonged hospitalization, increasing the treatment costs and also leading to poor

patient outcome in the form of increased morbidity and mortality [2].

During the few decades, all the efforts to combat MDR microorganisms were largely focused on Gram-positive bacteria. Unfortunately, the problems of MDR Gram-negative bacteria were not accompanied by advances in pertinent therapeutic options [6,8]. Thus, to prevent the world from reverting to the travails of preantibiotic era, it is now an opportune time to intensify attention towards Gram-negative resistance.

This study was carried out to determine the burden of XDR and PDR Gram-negative bacteria at a tertiary-care centre.

TABLE 2. Species distribution of extensively resistant and pandrug-resistant Gram-negative bacteria

Resistance or susceptibility	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Proteus spp.</i>	<i>Acinetobacter baumannii</i>	Other
Resistant to all antibiotics except tigecycline and colistin	18	28	2	—	16	3
Susceptible only to tigecycline	—	2	—	—	2	—
Susceptible only to colistin	—	—	29	—	1	—
Resistant to all antibiotics including tigecycline and colistin	—	2	3	5	—	1

Of 1240 Gram-negative bacteria isolated during the study period, 112 isolates (9%) were found to be resistant to all the antibiotics tested by Kirby-Bauer disc diffusion method. The most common isolate which was found to be resistant to all antibiotics tested was *P. aeruginosa*, followed by *K. pneumoniae*, *A. baumannii*, *E. coli* and *Proteus* spp. (Table 1).

It is clear from Fig. 1 that the most common sample from which these resistant isolates were obtained was urine (37.5%), followed by wound swab/pus (22.3%), tracheal aspirates (17.9%) and others. Most of the isolates were obtained from acute wards (42.9%) and ICUs (29.5%) followed by other wards (23.2%). It was interesting to note that five isolates (4.4%) were also obtained from patients attending OPD. The finding that these XDR isolates have been isolated from the OPD patients corroborates with other studies [3].

As there is no disc diffusion interpretive criteria in CLSI guidelines for drugs such as colistin and tigecycline, all 112 isolates were subjected to automated susceptibility testing by Vitek-2 (bioMérieux) using GN-AST cards, and MICs of various antibiotics was determined. The results of Vitek-2 were in concordance with Kirby-Bauer disc diffusion testing, as all the 112 isolates were found to be resistant to all the antibiotics tested by both methods.

The growing resistance among Gram-negative bacteria to commonly used antibiotics has led to the resurgence of the use of previously discarded antibiotics such as colistin as a last-resort treatment option. However, the use of colistin has its own disadvantages because it is a neurotoxic and nephrotoxic agent [9]. Despite the toxicity of this relatively old agent, colistin is frequently used to treat infections due to carbapenem-resistant Enterobacteriaceae. In most cases, colistin is the last viable effective option for the treatment of invasive bloodstream infections that are due to carbapenemase-producing Gram-negative bacteria. Overuse of colistin has recently led to the emergence of resistance to this lifesaving agent [9,10].

Tigecycline is a minocycline derivative belonging to the new class of antimicrobial drugs known as glycylcyclines. It was the first glycylcycline antibiotic to be approved by the US Food and Drug Administration in June 2005 [11]. It is a broad-spectrum antimicrobial drug with activity against many Gram-positive, Gram-negative and anaerobic pathogens and has been regularly prescribed as a part of combination schemes against carbapenem-resistant Enterobacteriaceae and also Carbapenem-resistant *A. baumannii* [12,13]. Unfortunately, tigecycline is not active against *P. aeruginosa* [11]. Despite some differences in the reported susceptibility breakpoints of this drug (1 or 2 mg/L), it has been shown in many surveillance studies that tigecycline presents good *in vitro* activity against many MDR and XDR Enterobacteriaceae and *A. baumannii* isolates [14].

Apart from the antibiotics tested by disc diffusion method, Vitek-2 also determined the MICs of tigecycline and colistin. Of these 112 isolates, 67 isolates were found to be susceptible to both tigecycline and colistin but were resistant to other antibiotics.

A total of 30 isolates were found to be susceptible to only colistin, out of which 29 were *P. aeruginosa* and one was *A. baumannii*. As mentioned earlier, in case of MDR Gram-negative organisms, the need for alternative treatments has led to the resurgence of colistin use. Although colistin has been shown to be effective for the treatment of a wide variety of infections, its use for treating infections caused by these three Gram-negative organisms has been impeded by occurrences of colistin resistance. Development of resistance to colistin is a serious concern [15]. In the present study, four isolates of *K. pneumoniae*, three isolates of *P. aeruginosa* and two isolates of *A. baumannii* were resistant to colistin, in addition to other antibiotics tested by disc diffusion, which is a cause for concern. As colistin is the last line of defense against these virulent pathogens, resistance to this antibiotic may have devastating effects if no other treatment options are available to combat the infection.

In the present study, two isolates of *K. pneumoniae* and one isolate of *A. baumannii* were resistant to tigecycline, which is an important finding. As *P. aeruginosa* is intrinsically resistant to tigecycline, its susceptibility testing was not evaluated by Vitek-2. Tigecycline resistance among Gram-negative bacteria such as *K. pneumoniae* and *A. baumannii* is rare but has been reported in a few studies [16,17]. Four isolates were susceptible to only tigecycline, of which two were *K. pneumoniae* and two were *A. baumannii*.

The most important finding of our study was that a total of six isolates were resistant to both colistin and tigecycline, out of which three were *P. aeruginosa*, two were *K. pneumoniae* and one was *H. alvei*. *Proteus* spp. are intrinsically resistant to tigecycline and colistin, so testing for these antibiotics against *Proteus* spp. was not done by Vitek-2. Thus, 101 (8.1%) of 1240 isolates were nonsusceptible to two or fewer class of antimicrobials and were XDR, which is less than that reported by Bajpai et al. [6], who reported 12% XDR. A total of 11 isolates (0.9%) were PDR, being resistant to all antimicrobials including colistin and tigecycline. This prevalence of 0.9% PDR Gram-negative bacteria in the present study is also less than that reported by Bajpai et al. [6], who reported 2.1% of bacteria to be PDR.

Conclusion

The prevalence of extensive drug resistance and PDR among Gram-negative bacterial isolates was 8.1% and 0.9%,

respectively, which is disturbingly high. These findings are alarming because infections with these XDR and PDR Gram-negative bacteria leave clinicians with no treatment options, leading to increased morbidity and mortality. The other important finding of this study is that few XDR isolates were obtained from patients attending OPD. This is a cause for concern, as it can be deduced that these resistant bacteria have disseminated in the community. The growing resistance of Gram-negative organisms and the emergence of XDR and PDR strains need to be curbed. This situation warrants the implementation of an efficient infection control program and intensive surveillance of antimicrobial resistance of Gram-negative bacteria so as to establish a rational antibiotic stewardship program for the sustainable management of such infections.

Conflict of interest

None declared.

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