INTRODUCTION
Antimicrobial resistance poses a major threat to public health and makes therapeutic decisions more challenging. India carries one of the largest burdens of drug-resistant pathogens worldwide [1]. Emergence of their multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative bacilli (GNB) so that it can become a good alternative as empirical treatment for severe sepsis.

Methods: Identification and antibiotic sensitivity testing of the GNB isolated from the clinical samples were done using the VITEK-II system in a tertiary care hospital, Kolkata. MDR and XDR strains were selected by their definitions and molecular characterization was done by multiplex polymerase chain reaction. The minimum inhibitory concentration (MIC) value of arbekacin was detected by the E-test strip and compared with other aminoglycosides.

Results: A total of 140 drug-resistant strains including ESBL- and carbapenemase-producing GNB were selected for the study. Arbekacin showed reduced values of MIC$_{50}$ and MIC$_{90}$ compared to other aminoglycosides for most of the drug-resistant GNB.

Conclusion: Hence, in this drug-resistant era, arbekacin with the advantage of a single daily dose can be used as an empirical choice in severe sepsis as monotherapy or in combination with other antibiotics such as colistin or polymyxin to fight against MDR and XDR bugs.

Keywords: Aminoglycoside, Arbekacin, Gram-negative bacilli, Multidrug resistant, Extensively drug resistant.

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References
1. S. S. S. S. and S. S., "Drug-resistant GNB was done from different samples (urine, respiratory samples, pus, and blood) by standard microbiological procedure and identification up to the species level was done using VITEK-GN cards (bioMérieux India Private Limited)."
2. A. M. A. and A. M., "Antimicrobial susceptibility testing was performed in the VITEK-2 system using AST-GN280 and AST-GN281 susceptibility cards and interpreted according to the Clinical and Laboratory Standards Institute recommendations. P. aeruginosa ATCC 27853 and Escherichia coli ATCC 25922 were used as quality control strains.
3. I. C. and I. C., "Selection for MDR and XDR strains from the isolated GNB was based on the definition of MDR (acquired non-susceptibility to at least one agent in three or more antimicrobial categories) and XDR (non-susceptibility to at least one agent in all but two or fewer antimicrobial categories) and XDR Gram-negative bacilli (GNB) was not evaluated. Hence, the objective of the study was to see the in vitro activity of arbekacin against MDR and XDR GNB isolated from different clinical samples.
4. M. G. and M. G., "Molecular characterization of MDR and XDR Gram-negative strains: The presence of carbapenemase-encoding genes from the selected MDR and XDR GNB was determined by multiplex PCR using primers (ReadyMade™ Primers, Integrated DNA Technologies) (Table 1) targeting blaVIM, blaIMP, blaKPC, blaOXA-48, and blaNDM (Fig. 1).
5. E. S. and E. S., "ESBL production was determined by placing ceftazidime (CAZ 30 µg) disks with or without clavulanic acid (CA 10 µg) on MHA plate. After overnight incubation, if there was an augmentation of 2.5 mm in the inhibitory zone diameter of CAZ-CA in comparison to CAZ alone, ESBL by that strain was phenotypically confirmed."
The minimum inhibitory concentration (MIC) of arbekacin against MDR and XDR Gram-negative bacterial isolates
To calculate the MIC of arbekacin by E-test strip, a lawn culture is made by the organism to be tested, over a Mueller-Hinton agar plate and the arbekacin E-test strip was applied onto it. After overnight incubation, the reading of MIC was taken as the value at the point where ellipse intersects the scale (Fig. 2).

RESULTS
Identification of the isolates from clinical samples
A total of 1276 GNB were isolated during the study period. Of them, 526 were E. coli, 314 were Klebsiella pneumoniae, 24 were Enterobacter cloacae, 48 were Proteus spp., 190 were P. aeruginosa, and 174 were Acinetobacter spp.

Among them, 140 representatives drug-resistant GNB were selected according to their antibiotic resistance pattern (Table 2). Of them, 30 were E. coli, 55 were K. pneumoniae, 12 were E. cloacae, 3 were Proteus spp., 24 were P. aeruginosa, and 16 were Acinetobacter spp. Arbekacin breakpoints were used according to Lee et al., 2007 [13], as susceptible ≤4 µg/mL; intermediate 8 µg/mL; and resistant ≥16 µg/mL.

Antimicrobial susceptibility testing
Arbekacin showed 50% resistance to E. coli, 54.55% resistance to Klebsiella spp., 33.34% resistance to E. cloacae, 66.67% resistance to Proteus spp., 20% resistance to Pseudomonas spp., and 50% resistance to Acinetobacter spp. (Table 3).

According to the resistance pattern of the selected Gram-negative isolates for the study, 50 strains are MDR and 90 strains are XDR (Table 4).

Among 50 MDR Gram-negative strains, 34 (68%) are sensitive and 16 (32%) are resistant to arbekacin, whereas among 90 XDR Gram-negative strains, 43 (47.78%) are sensitive to arbekacin and 47 (52.23%) are resistant.

The MIC of arbekacin in MDR and XDR Gram-negative strains
MIC\textsubscript{50} and MIC\textsubscript{90} of arbekacin for E. coli were 1.5 and 16, for K. pneumoniae 16 and 64, for E. cloacae 0.75 and 8, for Proteus spp. 24 and >128, for Pseudomonas aeruginosa 1.5 and 8, and Acinetobacter spp. 4 and 64, respectively (Table 5).

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Table 1: Primer sequences and amplicon sizes

| PCR name         | Targeted gene | Primer sequence (5’to3’) | Amplicon size |
|------------------|---------------|--------------------------|---------------|
| CARBA            | blaNDM        | Forward ACT TGG CCT TGC TGT CTT T | 603 bp        |
|                  |               | Reverse CAT TAG CCG CTT CAT TGA T |               |
|                  | blaVIM        | Forward TGT CCG TGA TGG TGA TGA G T | 437 bp        |
|                  |               | Reverse ATT CAG CCA GAT CGG CAT C |               |
|                  | blaIMP        | Forward ACA YGG YTT RGT DGT KCT TGG | 387 bp        |
|                  |               | Reverse GGT TTA AYA AAR CAA CCA CC |               |
|                  | blaKPC        | Forward TCG CCG TCT AGT TCT GCT TTC TTG | 353 bp        |
|                  |               | Reverse ACA CCT CCG CCA CCG TCA T |               |
|                  | blaOXA-48     | Forward ACG GGT GTA TTA GCC CCT TTA TCG | 265 bp        |
|                  |               | Reverse CAT CCT TAA CCA CGC CCA AAT C |               |
|                  |               | Forward CCC GTA AGA TGG TTC TAC G | 330 bp        |
|                  |               | Reverse TAC TGG GCA GGT GCT TCA GA |               |

PCR: Polymerase chain reaction, CARBA: Carbapenemase, NDM: New Delhi metallo-β-lactamase, VIM: Verona integron-encoded metallo-β-lactamase, IMP: Imipenemase, KPC: Klebsiella pneumoniae carbapenemase, OXA: Oxacillinase

Table 2: Isolation and identification of selected MDR and XDR GNB from different sources

|                  | Urine | Wound swab | Blood | Sputum and endotracheal aspirates | Total |
|------------------|-------|------------|-------|-----------------------------------|-------|
| Escherichia coli | 10    | -          | 20    | -                                 | 30    |
| Klebsiella pneumoniae | 30     | 5          | 15    | 5                                 | 55    |
| Enterobacter cloacae | 10   | 2          | -     | -                                 | 12    |
| Proteus spp.     | 1     | 2          | -     | -                                 | 3     |
| Pseudomonas aeruginosa | 5       | 9          | 5     | 5                                 | 24    |
| Acinetobacter baumannii | -   | 5          | -     | 11                                | 16    |
| Total            | 56    | 23         | 40    | 21                                | 140   |
Arbekacin is a unique aminoglycoside with a favorable pharmacodynamics, evidenced by the MIC value of arbekacin being lower than that of amikacin for Enterobacter spp., and gentamicin for Pseudomonas aeruginosa (MIC of arbekacin was 5.33 times lower than that of amikacin, and 2 times lower than gentamicin). This study showed that MIC of arbekacin was 10.67 times lower than that of amikacin and gentamicin for E. coli, 4 times lower than amikacin, and 2 times lower than gentamicin in Klebsiella spp., 1.33 times lower than amikacin, and 2.67 times lower than that of gentamicin for Enterobacter spp. In the case of Pseudomonas spp., MIC of arbekacin was 5.33 times lower than that of amikacin and gentamicin. Therefore, the MIC value of arbekacin was high in Klebsiella (MIC = 16, MIC ≤ 64) and Proteus (MIC = 24, MIC ≤ 128). In these cases, we might consider the combination therapy with polymyxins/tigecycline or carbapenem (if carbapenem was sensitive) [14]. Antibiotic combination therapy was suggested to improve clinical efficacy, and the Japanese Society of Chemotherapy and the Japanese Society of Therapeutic Drug Monitoring experts recommend 5.5–6.0 mg/kg body weight to reach the target concentration [19]. The pharmacokinetics in healthy volunteers monitoring experts recommend 5.5–6.0 mg/kg body weight to reach the target concentration [18], unlike other aminoglycosides which will help us to use it against pneumonia and other respiratory diseases. Again, simultaneous Gram-positive and Gram-negative coverage with a single daily dose are the advantage of arbekacin and it can be used as an empirical choice in severe sepsis. To interpret its favorable pharmacodynamics, we might consider the combination therapy with polymyxins/tigecycline or carbapenem (if carbapenem was sensitive) [14]. Antibiotic combination therapy was suggested to improve clinical efficacy and the Japanese Society of Chemotherapy and the Japanese Society of Therapeutic Drug Monitoring experts recommend 5.5–6.0 mg/kg body weight to reach the target concentration [19]. The pharmacokinetics in healthy volunteers with normal renal function did not change on 400 and 600 mg single dose and the total clearance does not decrease at a high dose [8].

**DISCUSSION**

The increased prevalence of MDR and XDR GNB along with their rapid spread is a matter of concern in modern medicine. In our study period over 4 months, we have selected 140 drug-resistant strains including ESBL and carbapenem-producing GNB which were representative of MDR and XDR strains among the total of 1276 isolates. We compared the resistance pattern of arbekacin with other aminoglycosides (amikacin and gentamicin) and other potent antimicrobial drugs with these selected isolates. Arbekacin showed a better sensitivity profile than penicillin, cephalosporin, other aminoglycosides, fluoroquinolones, β-lactam-β-lactamase inhibitors, and carbapenems. Polymyxin B and tigecycline showed better sensitivity than arbekacin. In Proteus spp., the sensitivity of arbekacin was comparable with other aminoglycosides.

This study showed that MIC of arbekacin was 10.67 times lower than that of amikacin and gentamicin for E. coli, 4 times lower than amikacin, and 2 times lower than gentamicin in Klebsiella spp., 1.33 times lower than amikacin, and 2.67 times lower than that of gentamicin for Enterobacter spp. In the case of Pseudomonas spp., MIC of arbekacin was 5.33 times lower than that of amikacin and gentamicin. However, the MIC value of arbekacin was high in Klebsiella (MIC = 16, MIC ≤ 64) and Proteus (MIC = 24, MIC ≤ 128). In these cases, we might consider the combination therapy with polymyxins/tigecycline or carbapenem (if carbapenem was sensitive) [14]. Antibiotic combination therapy was suggested to improve clinical efficacy and the Japanese Society of Chemotherapy and the Japanese Society of Therapeutic Drug Monitoring experts recommend 5.5–6.0 mg/kg body weight to reach the target concentration [19]. The pharmacokinetics in healthy volunteers with normal renal function did not change on 400 and 600 mg single dose and the total clearance does not decrease at a high dose [8].

**Table 3: Resistance (%) pattern of the isolates selected for the study**

| Strain                  | AMC | 3rd gen Ceph | 4th gen Ceph | CIP | 3rd gen Ceph | 4th gen Ceph | PTZ | ETP | IPM | MRP | TGC |
|-------------------------|-----|--------------|--------------|-----|--------------|--------------|-----|-----|-----|-----|-----|
| Escherichia coli (30)   | -   | 100%         | 100%         | -   | 100%         | 100%         | -   | 100%| 100%| 100%| -   |
| Klebsiella pneumoniae (55) | - | 100%   | 100% | - | 100% | 100% | - | 100% | 100% | 100% | - |
| Enterobacter cloacae (12) | - | 100%   | 100% | - | 100% | 100% | - | 100% | 100% | 100% | - |
| Proteus spp. (13)       | -   | 100%         | 100%         | -   | 100%         | 100%         | -   | 100%| 100%| 100%| -   |
| Acinetobacter spp. (16) | -   | 100%         | 100%         | -   | 100%         | 100%         | -   | 100%| 100%| 100%| -   |

**Table 4: Number of MDR and XDR strains selected for the study**

| Strain                  | MDR strains | XDR strains |
|-------------------------|-------------|-------------|
| Escherichia coli        | 10          | 20          |
| Klebsiella pneumoniae   | 20          | 35          |
| Enterobacter cloacae    | 9           | 3           |
| Proteus spp.            | 0           | 3           |
| Pseudomonas aeruginosa  | 11          | 13          |
| Acinetobacter baumannii | 0           | 16          |

MDR: Multidrug resistant, XDR: Extensively drug resistant.
If the dose of arbekacin is formulated as 4 times the normal dose with dose spacing of 72 h, it will have the following benefits: (i) The dose will cross 4 times the normal MIC which is well above minimum bactericidal concentration (MBC) and for that the chance of cross-resistance or resistant mutant will be nil. (ii) If the dose is 4 times increased, it will be far less than the toxic dose and therefore safe. The renal-related adverse drug reactions of arbekacin are increased with a higher C trough [11]. The incidence of arbekacin induced nephrotoxicity was observed when it was administered at a total dose of over 5000 mg [20]. Moreover, with a dose spacing of 72 h, the C trough will be lower. (iii) Outpatient antimicrobial therapy is possible in resource-limited settings. (iv) It can be cost effective and can be used in remote places where a basic sensitivity pattern is available.

Hence, in case of severe sepsis, we can plan the treatment in the following way – first, send the culture from all relevant sites and give arbekacin 800 mg (4 times the normal dose to attend the MBC). No antibiotics are needed for the next 72 h until the culture sensitivity report comes and plan the antibiotics accordingly. All other supportive measures are to be continued as per the sepsis protocol. The total cost of therapy will be less with a fair chance of patient survival.

CONCLUSION

Hence, in this drug-resistant era, arbekacin can be used as an empirical choice in severe sepsis as monotherapy or in combination with other antibiotics such as colistin or polymyxin to fight against MDR and XDR bugs. The favorable pharmacokinetics, pharmacodynamics, and spectrum (simultaneous Gram-positive and Gram-negative coverage) of the drug with the advantage of a single daily dose will make the antibiotic as a handy choice for the management of sepsis with least time to the thermometer to needle even in resource-limited health-care settings in India.

AUTHORS’ CONTRIBUTIONS

Dr. Soma Sarkar – Data collection and manuscript writing, Dr. Dipankar Sarkar – Design the research study, Dr. Anjum Namhata – Preparation of manuscript, and Dr. Manideepa Sengupta – Editing of the manuscript as per journal guidelines.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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