DOI 10.18019/1028-4427-2020-26-4-544-547

# *Monitoring of gram-negative bacteria and antibiotic resistance in osteomyelitis*

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**Introduction** There is an urgent need for a surveillance system of regular monitoring of specific bacteria inducing various types of osteomyelitis to identify resistant isolates and optimize the use of antibiotics. **Objective**: monitoring of specific gram-negative bacteria and analysis of the antibiotic resistance of the strains isolated from osteomyelitis patients over a three-year period. **Results and discussion** *P. aeruginosa* was the first most common pathogen among gram-negative microorganisms isolated from the patients between 2017 and 2019. Prevalence of the isolates identified in 2019 decreased by 9.6 % as compared to 2017. Next frequently encountered clinical isolates were *Enterobacter sp., Acinetobacter sp., Klebsiella sp*. There was a two-fold increase in *K. pneumoniae* strains isolated in 2019. Analysis of antibiotic susceptibility testing data revealed multiresistance of the *Acinetobacter sp*. strains in 2019 despite the total decrease in resistant isolates in 2017 and 2018. Among non-fermenting gram-negative rods, the species being resistant to imipenem were shown to increase by 5.4 times. Overall antibiotic resistance was on rise. Increased antimicrobial resistance to beta-lactam antibiotics also combined with BLaC inhibitors was observed in *Enterobacteriaceae* population. Meropenem was found to be effective against most bacteria with growing drug resistance observed as compared with recent years. The antibiotic resistance profiles of *Klebsiella sp*. strains appeared to be high at antimicrobial testing. **Conclusion** Diverse bacterial morphology of gram-negative species and increasing proportion of drug-resistant strains isolated in osteomyelitis cases have necessitated regular monitoring of multiresistant clinical isolates for adjustment of empirical antibiotic therapies.

**Keywords**: osteomyelitis, gram-negative bacteria, antibiotic resistance, beta-lactam antibiotics, multi-drug resistance

## INTRODUCTION

Timely and accurate detection of microbial pathogens, debridement and administration of antibiotics are an essential part of osteomyelitis management [1, 2, 3]. Antimicrobial prescription would rely on pharmacological characteristics including bactericidal effects, the possibility of higher concentrations to be accumulated in the bone and soft tissues, long-term administration of antibiotics and safe use [1, 4, 5]. Therapeutic options for osteomyelitis caused by gram-negative organisms of the *Enterobacteriaceae* family expressing plasmid-mediated β-lactamases are limited because these organisms are usually resistant to penicillins and cephalosporins [4, 6, 7]. Non-fermenting gram-negative bacteria (NFGNB) are normally multiresistant [7] due to primary non-susceptibility to antimicrobial agents and acquired mechanisms of resistance [4, 7, 8]. Carbapenems and BLaC inhibitor combinations are among the last-resort antimicrobial agents for gram-negative rods [4, 9]. The clinical effect of antibiotics against organisms can be very limited because of the global emergence of multi-drug resistant isolates that are complicating the treatment of chronic osteomyelitis. There is an urgent need for a surveillance system of regular monitoring of specific bacteria inducing various types of osteomyelitis to identify resistant isolates and optimize the use of antibiotics.

**Objective**: monitoring specific gram-negative bacteria and analysis of the antibiotic resistance of the strains isolated from osteomyelitis patients over a three-year period.

## MATERIAL AND METHODS

The review included gram-negative microorganisms primarily isolated from wounds and sinuses of patients who received treatment at the clinic of infection osteology RISC RTO between 2017 and 2019. The pure culture was grown using routine techniques based on current guidelines of clinical microbiology. The NBC 44 gram-negative panels (WalkAway-40 Plus, «Siemens») were used to identify microorganisms and test the susceptibility of antibiotics used to treat osteomyelitis in clinical settings. Statistical analysis was performed with *Attestat* Version 13.0 statistic software package. The data obtained were summarized as the arithmetic mean ± standard deviation (M ± m).

Shipitsyna I.V., Osipova E.V., Leonchuk D.S., Sudnitsyn A.S. Monitoring of gram-negative bacteria and antibiotic resistance in osteomyelitis. *Genij Ortopedii*, 2020, vol. 26, no 4, pp. 544-547. DOI 10.18019/1028-4427-2020-26-4-544-547

## RESULTS

In 2017, a total of 689 bacterial isolates of gram negative rods, representing 379 NFGNB and 310 species of *Enterobacteriaceae* family, were available for this study. *Pseudomonas aeruginosa* was the first most common pathogen (292 isolates) isolated from clinical samples. Next frequently encountered clinical isolates were *Enterobacter sp*., *Acinetobacter baumannii, Klebsiella pneumoniae, Proteus sp., Escherichia coli*, species of Enterobacteriaceae population and nonfermenters (Table 1). A total of 664 bacterial isolates of gram negative rods were identified in 2018 with decreased proportion of *P. aeruginosa* strains by 4.5 %, *A. baumanni* by 19.4 %, *Proteus sp.* by 18.6 % and increased *K. pneumoniae* by 17.9 % and species of *Enterobacteriaceae* family by 44.4 %. Bacterial isolates of gram negative rods (662) identified in 2019 were similar to those identified in 2018. However, there still seemed to be some heterogeneity among strains identified in the years of study. There was a 39.7 % increase in *K. pneumoniae* strains isolated in 2019 as compared to 2018 and a 48.9 % increase in the strain as compared to 2017. There was a 20.0 % increase in *Proteus sp*. strains isolated in 2019 as compared to 2018.

In 2019, the sensitivity of the *Enterobacter sp*. and *Klebsiella sp*. strains to cefuroxime was 73.0 % and 90.0 %; to ceftazidime, 71.1 % and 75.2 %, respectively (Table 2). Overall antibiotic resistance was on rise during the three years. Antimicrobial resistance of *Proteus sp*. and *E. coli* isolates to cephalosporins ranged between 40 to 50 %. Ampicillin was not active against *Enterobacteriaceae* isolates. The three-year mean antimicrobial resistance was 94.6 ± 3.9 %; 95.1 ± 2.6 %; 57.6 ± 12.6 %; 80.5 ± 5.2 % for *Enterobacter sp*., *Klebsiella sp*., *Proteus sp*., *E. coli* isolates and other representatives of *Enterobacteriaceae* family, respectively. Meropenem was very active against *Enterobacteriaceae* in 2017 and 2018 showing 100 % efficacy against *E. coli* isolates in 2017 with resistance of *E. coli* strains increased to 12 % in 2019. The antimicrobial drug effect decreased by 54.1 % for *Enterobacter sp*. and by 41.4 % for *Klebsiella sp. Enterobacter sp., Klebsiella sp*. showed resistance against registered beta-lactam antibiotic amoxiclav that exhibited 35.1 % inhibition against the *Proteus sp*. isolates. The percentage of resistant *E. coli* strains decreased by 25.6 % in 2019 as compared to 2017. Amoxiclav resistant strains in the *Enterobacteriaceae* family constituted 65.8 ± 3.2 %. Gentamicin resistant strains of *Enterobacter sp*. decreased by 23.5 % in 2019 as compared to 2017. There was homogeneity among resistant strains of *Klebsiella sp., Proteus sp., E. coli* identified in 2017 and 2019 measuring 64.2 % and 42.2 %, respectively. Gentamicin resistant strains decreased in the *Enterobacteriaceae* family by 61.9 %. Ciprofloxacin resistant strains of *Enterobacter sp*. decreased by 56.9 % in 2017–2019. Ciprofloxacin appeared to be most resistant to strains of *Klebsiella sp*. with the resistance of 87.3 % recorded in 2019. Other bacterial isolates of *Enterobacteriaceae* population were shown to be less resistant to gentamicin and meropenem with the resistance of 10.7 % and 3.6 % recorded, respectively, in 2019.

Among NFGNB, the proportion of amikacin resistant strains of *P. aeruginosa* increased by 24.6 % in 2019 as compared to 2017. The antimicrobial resistance of *P. aeruginosa* isolates tested in 2019 ranged from 42.7 % to amikacin to 59.2 % to ceftazidime. The antimicrobial resistance of *Acinetobacter sp*. isolates decreased by 27.7 % to amikacin, 19.2 % to gentamicin and 13.3 % to ciprofloxacin in 2019 as compared to 2017. However, the proportion of resistant strains to the antibiotics tested increased by 50 %. Ceftazidime, cefepime and ciprofloxacin were shown to be most effective antimicrobial agents in our series. The resistance of other NFGNB to the antibiotics tested measured 42.9 ± 7.3 % in 2017–2019 with imipenem resistance increased by 81.3 %.

Table 1

| Microorganism | Bacterial isolates of gram negative rods, n | | |
|---|---|---|---|
| | 2017 | 2018 | 2019 |
| *P. aeruginosa* | 292 (42.4 %) | 279 (42.0 %) | 264 (39.9 %) |
| *A. baumannii* | 72 (10.4 %) | 58 (8.7 %) | 53 (8.0 %) |
| Nonfermenters | 15 (2.2 %) | 27 (4.1 %) | 21 (3.2 %) |
| *Enterobacter sp.* | 79 (11.4 %) | 82 (12.3 %) | 40 (6.0 %) |
| *K. pneumoniae* | 67 (9.8 %) | 79 (11.9 %) | 131 (19.8 %) |
| *Proteus sp.* | 59 (8.7 %) | 48 (7.3 %) | 60 (9.1 %) |
| *E. coli* | 57 (8.4 %) | 60 (9.0 %) | 60 (9.1 %) |
| Other representatives of *Enterobacteriaceae* population | 48 (6.7 %) | 31 (4.7 %) | 33 (4.9 %) |
| Total | 689 (100 %) | 664 (100 %) | 662 (100 %) |

Table 2

Antimicrobial resistance in Enterobacteriaceae family in 2017–2019, %

| Antimicrobial agent | Year | *Enterobacter sp.* | *Klebsiella sp.* | *Proteus sp.* | *E. coli* | Other representatives of *Enterobacteriaceae* |
|---|---|---|---|---|---|---|
| Amoxicillin | 2017 | 89.1 | 74.2 | 30.2 | 58.1 | 70.1 |
| | 2018 | 90.7 | 76.6 | 30.6 | 54.1 | 62.9 |
| | 2019 | 90.0 | **82.0** | 35.1 | **43.2** | 64.3 |
| Ampicillin | 2017 | 91.2 | 93.1 | 77.2 | 84.0 | 75.1 |
| | 2018 | 97.4 | 97.2 | 42.2 | 73.5 | 80.7 |
| | 2019 | 95.2 | 95.1 | **53.4** | **72.0** | **85.7** |
| Gentamicin | 2017 | 54.3 | 58.2 | 38.1 | 47.2 | 28.1 |
| | 2018 | 39.7 | 62.9 | 20.5 | 30.4 | 25.0 |
| | 2019 | **71.0** | 64.2 | 422 | 43.3 | 10.7 |
| Meropenem | 2017 | 11.1 | 31.1 | 2.0 | 0.0 | 10.2 |
| | 2018 | 15.1 | 32.8 | 4.0 | 1.7 | 3.9 |
| | 2019 | **24.2** | **53.1** | 2.1 | **12.0** | **3.6** |
| Cefotaxime | 2017 | 66.2 | 80.1 | 47.0 | 51.0 | 35.2 |
| | 2018 | 54.7 | 76.3 | 28.6 | 47.6 | 25.0 |
| | 2019 | 73.0 | **90.0** | 47.6 | 49.4 | 35.7 |
| Ceftazidime | 2017 | 66.3 | 81.4 | 45.1 | 51.0 | 33.1 |
| | 2018 | 60.5 | 78.9 | 30.6 | 54.1 | 26.9 |
| | 2019 | 71.1 | 75.2 | 50.4 | 50.2 | 39.7 |
| Ciprofloxacin | 2017 | 67.4 | 78.2 | 62.0 | 63.1 | 40.3 |
| | 2018 | 40.7 | 74.3 | 28.6 | 38.5 | 26.9 |
| | 2019 | **29.0** | **87.3** | **53.7** | **43.3** | 32.1 |

Table 3

Antibiotic resistance of non-fermenting gram-negative bacteria (NFGNB) in 2017–2019, %

| Antimicrobial agent | Year | *P. aeruginosa* | *Acinetobacter sp.* | Other NFGNB |
|---|---|---|---|---|
| Amikacin | 2017 | 32.2 | 78.7 | 46.0 |
| | 2018 | 42.5 | 68.9 | 35.3 |
| | 2019 | **42.7** | 59.3 | 46.7 |
| Gentamicin | 2017 | 40.2 | 71.4 | 46.0 |
| | 2018 | 48.9 | 67.2 | 35.3 |
| | 2019 | 48.4 | 57.7 | 33.3 |
| Imipenem | 2017 | 38.4 | 54.2 | 8.1 |
| | 2018 | 50.7 | 65.6 | 23.5 |
| | 2019 | **52.3** | 57.5 | **43.4** |
| Meropenem | 2017 | 38.4 | 67.3 | 38.2 |
| | 2018 | 48.4 | 68.8 | 20.0 |
| | 2019 | **49.6** | 59.6 | **50.0** |
| Cefepime | 2017 | 51.4 | 78.0 | 31.2 |
| | 2018 | 58.7 | 81.9 | 17.6 |
| | 2019 | 58.8 | 72.3 | **50.0** |
| Ceftazidime | 2017 | 52.7 | 83.3 | 46.3 |
| | 2018 | 62.5 | 86.9 | 27.2 |
| | 2019 | 59.2 | 76.1 | 50.0 |
| Ciprofloxacin | 2017 | 57.3 | 83.0 | 46.1 |
| | 2018 | 57.1 | 86.9 | 33.3 |
| | 2019 | 54.4 | 70.2 | **66.7** |

## DISCUSSION

*P. aeruginosa* was the first most common pathogen among gram-negative microorganisms isolated from osteomyelitis patients between 2017 and 2019. Prevalence of the isolates identified in 2019 decreased by 9.6 % as compared to 2017. Next frequently encountered clinical isolates were *Enterobacter sp., Acinetobacter sp., Klebsiella sp*. There was a two-fold increase in *K. pneumoniae* strains isolated in 2019.

Analysis of antibiotic susceptibility testing data revealed multiresistance of the *Acinetobacter sp*. strains in 2019 despite the total decrease in resistant isolates in 2017 and 2018. Among non-fermenting gram-negative rods, the species being resistant to imipenem were shown to increase by 5.4 times. Overall antibiotic resistance was on rise. Increased antimicrobial resistance to beta-lactam antibiotics also combined with BLaC inhibitors was observed in *Enterobacteriaceae* population. Meropenem was found to be effective against most bacteria with growing drug resistance observed and compared with recent years.

The antibiotic resistance profiles of *Klebsiella sp*. strains appeared to be high at antimicrobial testing.

According to current literature and from our own experience, the importance of multidrug resistant gram-negative bacilli has become evident more recently, as being causative agents of osteomyelitis due to their antimicrobials susceptibility pattern for *Acinetobacter sp., Enterobacter sp., Klebsiella sp*. [10, 11, 12]. Among the most common types of antibiotics used for empirical treatment of osteomyelitis are beta-lactams [1, 4, 5]. The resistance of bacterial isolates to beta-lactam antibiotics is due principally to the production of beta-lactamases [4]. It is well known that many beta-lactamases are induced by exposure to beta-lactam antibiotics [4]. Extended-spectrum beta-lactamases (ESBLs) spread among *Enterobacteriaceae* and have been observed in NFGNB, P. *aeruginosa, A. baumannii*, in particular [7, 13]. Imipenem and meropenem are stable to hydrolysis by ESBLs [4]. An increase in the number of antimicrobial resistance strains in our series can be suggestive of the co-selection of bacteria with different mechanisms of resistance.

## CONCLUSION

Diverse bacterial morphology of gram-negative species and increasing proportion of drug-resistant strains isolated in osteomyelitis cases have necessitated regular monitoring of multiresistant clinical isolates for adjustment of empirical antibiotic therapies.

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Received: 29.05.2020

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