Prevalences of Antibiotic-Resistant and Multidrug Resistant Bacteria in Urine Cultures from Inpatients with Spinal Cord Injuries and Disorders: An 8-Year, Single-Center Experience

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Abstract

Background

Patients, especially inpatients, with spinal cord lesions and disorders (SCI/D) have an elevated risk of recurrent urinary tract infections with multidrug resistant (MDR) bacteria. This study evaluated antimicrobial resistance and the prevalence of multidrug resistance and determined the risk factors for multidrug resistance.

Methods

In this retrospective cohort study, urine culture results were used to calculate the antimicrobial resistance rate and the incidence of infection with MDR bacteria in the SCI/D population. MDR was defined as acquired nonsusceptibility to at least one agent from three or more antimicrobial categories. The cohort included 402 inpatients from 2013 to 2020, with 1385 urine isolates. We included only the first isolate, and duplicate isolates, defined as positive cultures of the same strain within 14 days, were excluded from the evaluation.

Results

The most common MDR strains were *Klebsiella spp.* (29%) and *Escherichia coli* (24%). MDR isolates were detected in 50% of the samples and extended spectrum beta-lactamase (ESBL)-producing isolates in 26%, while carbapenem resistance was found in 0.1%. Significantly higher rates of infection with MDR bacteria were identified in the groups of patients with indwelling urethral/suprapubic catheters (p=0.003) and severity score C1-C4/AIS A-C (p=0.01). We identified age (OR: 0.99, 95% CI; 0.98-0.99, p=0.000), male sex (OR: 1.55, 95% CI; 1.16-2.06, p=0.003), management with urethral/suprapubic catheters (OR: 2.76, 95% CI; 2.04-3.74, p=0.000), and spontaneous voiding (OR: 1.84, 95% CI; 1.03-3.29, p=0.038) as independent predictors of multidrug resistance in our study population.

Conclusions

We identified a high antibiotic resistance rate and an increasing prevalence of infection with MDR bacteria in the SCI/D inpatient population. Particular attention should be given to bladder management, with an emphasis on minimizing the use of indwelling catheters.

Background

Urinary tract infection (UTI) is very common in patients with spinal cord injury and disorders (SCI/D). Positive urine culture was reported in 50-75% of patients [1]. In general, each SCI/D patient had 2.5 UTIs

per year [2]. UTIs have been shown to be one of the most common complications of long-term treatment in SCI/D patients [3].

Increased bacterial resistance, especially multidrug resistance to antimicrobial agents, is now a major public health problem worldwide. Resistance to third-generation cephalosporins in *Escherichia coli* and *Klebsiella pneumoniae* is growing rapidly, primarily due to the production of extended-spectrum beta-lactamases (ESBLs), which are often associated with resistance to other antibiotics. Patients with multidrug resistant (MDR) *E. coli* and *K. pneumoniae* are often treated with carbapenems, but the number of carbapenem-resistant *Enterobacteriaceae* (CRE) isolates is increasing. In 2018, more than half of the *E. coli* isolates and more than one-third of the *K. pneumoniae* isolates were resistant to at least one antimicrobial group, and combined resistance was common as well [4]. The increase in vancomycin-resistant isolates of *Enterococcus faecium* (VRE) is also a problem [4].

SCI/D patients repeatedly access health care and have a higher level of exposure to antibiotics, which increases the risk of infection with and colonization by MDR strains, especially gram-negative bacteria (GNB). Infection with MDR strains is associated with much worse outcomes, a prolonged length of stay, increased morbidity and mortality, and a greater risk of impaired kidney function and urolithiasis, especially infectious urolithiasis [5–8]. This increase in infections with MDR bacteria is thus rapidly becoming a problem, especially in the inpatient setting.

In routine clinical practice, antimicrobial therapy should generally be deployed at the time of the development of clinical signs of a UTI. To use empirical treatments, which can be adjusted and targeted after receiving the results of antimicrobial susceptibility testing (AST), it is necessary to have an overview of the current epidemiological situation regarding uropathogens and resistance rates to antimicrobial agents.

Our study was performed to assess the resistance to the tested antimicrobial agents and the prevalence of infection with MDR strains in the SCI/D population and to identify the risk factors for infection with MDR strains.

Methods

Study population

This was a retrospective cohort study focused on antimicrobial drug resistance and infection with MDR bacteria in the inpatient SCI/D population. We included patients hospitalized for SCI/D in the spinal care ward from 1 January 2013 to 31 December 2020. We obtained data from the electronic medical records (EMRs) and the central database of the microbiology department. A total of 402 adult patients were enrolled, and six patients were excluded from the evaluation (four did not have SCI/D, two had incomplete data). A total of 396 patients were evaluated (303 men and 93 women); 1101 urine samples were taken, from which 1385 bacterial isolates were obtained. There was a mean of 2.5 urine samples per patient. One, two and three urine samples were taken from 140, 103 and 64 patients, respectively, and ≥ 4 urine

samples were taken from 89 patients. At the time of their first UTI, 55% of the patients were managed with an indwelling urethral catheter (UC), 9% with a suprapubic catheter (SC), 23% with clean intermittent catheterization (CIC), and 13% spontaneous voiding (SV). The detailed characteristics are given in Table 1.

Urine specimen collection

In patients with spontaneous voiding, we used 5 ml of clean-catch midstream urine; in patients on the CIC regimen, urine collected from the catheter was used. In patients managed with UC/SC, we used urine collected after catheter replacement. Urine collection was performed when clinical symptoms of UTI were observed, if UTI was suspected or for routine purposes.

Urine culture

The collected urine samples were inoculated on chromogenic agar UriSelect4® (Bio-Rad, France) within two hours. Samples taken outside working hours were stored at 2-8 °C according to the preanalytical standards. An evaluation was performed after 18-24 hours of aerobic incubation at 35 ± 2 °C. Identification of bacterial isolates was performed according to colony morphology, Gram staining, and MALDI TOF MS® mass spectrometry (Bruker, Daltonics, Germany). We considered a sample with a growth of $\geq 10^3$ colony-forming units/mL of primary pathogens to be positive.

Antimicrobial susceptibility testing (AST)

The antibiotic disk diffusion method was used in accordance with the Guidelines and breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [9]. AST was performed on Mueller-Hinton agar (Bio-Rad, France) using antibiotic disks (Bio-Rad, France). The evaluation was performed after 16-20 hours of incubation at 36 °C. The measured inhibition zones were evaluated according to the EUCAST standards as susceptible, intermediate (changed to “susceptible - increased exposure” starting in 2019), or resistant [9].

Only the first bacterial isolate per patient was included in the protocol; a duplicate isolate was defined as a positive culture of the same isolate obtained within 14 days of the initial isolate. Different isolates were considered different individual isolates. Polymicrobial isolates were not included when it was impossible to identify the individual components.

In accordance with the European Center for Disease Prevention and Control, MDR bacteria were defined as those with acquired nonsusceptibility to at least one agent in three or more antimicrobial categories. Extensively drug-resistant (XDR) bacteria were defined as those with nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., the bacterial isolates remained susceptible to only one or two antimicrobial categories). Pandrug resistant (PDR) bacteria were defined as those with nonsusceptibility to all agents in all antimicrobial categories [10].

Enterobacteriaceae strains resistant to amoxicillin-clavulanic acid and/or piperacillin-tazobactam and/or cefotaxime and/or meropenem were further tested using the AmpC & ESβL Detection Discs® kit (MAST, France) and were confirmed or not confirmed as being ESBL-producing strains. Strains of *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter spp.* resistant to meropenem in patients with positive β Carba Test® (Bio-Rad, France) results for carbapenemase-producing *Enterobacteriaceae* strains, were assessed for carbapenemase production in the National Reference Laboratory for Antibiotics (SZÚ, Prague, CZ) by MALDI TOF and PCR. *S. aureus* strains resistant to cefoxitin and oxacillin were considered strains of methicillin-resistant *Staphylococcus aureus* (MRSA). Strains of *E. faecalis* and *E. faecium* resistant to vancomycin were classified as vancomycin-resistant (VRE).

Study endpoints

- The primary objectives were to evaluate the resistance to the tested antibiotics, assess occurrence of multidrug resistance and determine the risk factors for the development of multidrug resistance.
- The secondary objectives were to evaluate the incidences of ESBL resistance, carbapenem resistance, vancomycin resistance, methicillin-resistant *Staphylococcus aureus*, extensive drug resistance and pandrug resistance.

Statistical methods

We used the mean, standard deviation, median and quartile values to describe continuous variables. To determine if there were the same number of patients infected with MDR and non-MDR bacteria within each category stratified by the variables sex, etiology, bladder management, severity of injury, time since injury, and urinary culture, we used the chi-square goodness-of-fit test. Univariate and multivariate logistic regression were used to determine the independent predictors of multidrug resistance. We present the results as adjusted odds ratios (ORs) with 95% confidence intervals (CIs). A significance level of 5% was used for all statistical tests. We used SPSS 18 statistical software (IBM, IL, USA) for the statistical analysis. When the term significance is used in the text below, it means statistical significance.

Results

During the study period, we evaluated the results of the urine culture and AST of 1385 bacterial isolates. Gram-negative bacteria were the most common (1191, 86.8%), and the rest were gram-positive cocci. The most common strains were *Klebsiella spp.* (402, 29%), *E. coli* (329, 24%), *P. aeruginosa* (180, 13%), *E. faecalis* (174, 12%), and *P. mirabilis* (133, 10%); other strains were much less common (Table 2).

The overall occurrence of multidrug resistance in the cohort was 50% (Table 2). The strains that were 100% MDR were *P. stuarti*, *M. morganii*, and *P. vulgaris*. In total, 27% of the strains were XDR, most of which were *Klebsiella spp*. (258, 64%), *P. stuarti* (21, 41%), *P. aeruginosa* (35, 19%). PDR strains accounted for 1%. ESBL resistance was found in 26%. The most common producers of ESBL were *Klebsiella spp.* (255, 63%) and *P. stuarti* (28, 55%). CPE resistance was identified in only 2 strains of *P. aeruginosa*. We did not observe VRE strains or MRSA.

The proportions of MDR strains over the study period are shown in Fig. 1. MDR strains of *P. vulgaris* became increasingly common, while MDR strains of other species remained stable. As shown in Table 3, we found that MDR strains were significantly more common in the group of patients managed with UC/SC (p=0.003). MDR strains were identified significantly less often in women (p=0.006) and those managed with CIC (p=0.000).

An overview of the rates of resistance of the isolates to the tested antibiotics is shown in Table 4. The results are based on AST. The resistance rates of isolates accounting for <1% are not listed in the table. The total levels of resistance to aminopenicillins and amoxicillin-clavulanic acid were extremely high at 70% and 48%, respectively. The total level of resistance to piperacillin-tazobactam was 35%. The total level of resistance to cefuroxime was 52%, while the levels of resistance to the third- and fourth-generation cephalosporins cefotaxime, ceftazidime and cefepime were 43%, 22% and 20%, respectively. The overall level of resistance to ciprofloxacin was also high at 49%. Among aminoglycosides, the overall rate of resistance was 38% for gentamicin and only 8% for amikacin. We identified a very low meropenem resistance rate (4%). The rate of resistance to sulfamethoxazole-trimethoprim was 63%. None of the enterococci were resistant to vancomycin.

We detected low resistance (<10%) only to meropenem and amikacin.

We identified the specific risk factors associated with the isolation of MDR strains. Based on univariate analysis, we identified four variables associated with the identification of MDR strains, namely, age, sex, bladder management and severity of the injury (Table 5). We used the enter method of multivariate logistic regression (Table 6) to identify the variables independently associated with the identification of MDR strains. Age (OR: 0.99, 95% CI; 0.98-0.99, p = 0.000), male sex (OR: 1.55, 95% CI; 1.16-2.06, p = 0.003), UC/SC bladder management (OR: 2.76, 95% CI; 2.0-3.74, p = 0.000), and SV bladder management (OR: 1.84, 95% CI: 1.03-3.29, p = 0.038) were found to be independent predictors of the isolation of MDR strains in our study population.

Discussion

Our work provides an overview of urine culture results and AST in spinal ward patients over eight years. We evaluated all positive urinary findings to obtain an overview of the rates of antibiotic resistance, enzymatically conditioned resistance, and multidrug resistance. Knowledge of these parameters is important for the empirical use of antimicrobial therapy before the final results of AST are obtained. Many infected SCI/D patients are in critical condition, and knowledge of the epidemiological data and the estimated resistance rates can affect the success of empirical antibiotic therapy. Infection with MDR strains increases morbidity and mortality, increases rehospitalization, prolongs the length of stay, and has a significant effect on the cost of treatment [11].

Because the primary objective was to determine the prevalences of uropathogens and the rates of resistance, including multidrug resistance, we did not take into account whether these infections were

symptomatic UTIs or asymptomatic bacteriuria. Additionally, a detailed assessment of this aspect would be error-prone given the retrospective nature of the study, which was based on data from the EMRs.

We used the currently valid definition of multidrug resistance, which was adopted based on the consensus reached by an international expert panel in 2011 [10]. These guidelines established epidemiologically significant categories of antibiotics for each group of bacteria and defined MDR bacteria as those with nonsusceptibility to at least one agent in ≥ 3 antimicrobial categories. Most of the studies conducted before this consensus used a different definition of MDR bacteria, which was usually less strict. Therefore, it is difficult to compare our results with those of many previously published studies.

In our study, which exclusively involved inpatients, 50% of the isolates were MDR bacteria, and the proportion of XDR bacteria was relatively high (27%). Fitzpatrick et al. demonstrated that 36.1% of GNB isolated from urine were MDR strains, one-fifth of which were obtained from outpatients [12]. The most common uropathogens were *E. coli* (27%), *K. pneumoniae* (16%) and *P. aeruginosa* (17.3%). Among the resistant pathogens, they observed a significant shift from gram-positive cocci to GNB at 9 years of follow-up. Significant geographical differences in MDR bacteria were also observed in the study. The results of other studies in the SCI/D population have shown the prevalence of MDR to be 60.7%, 41.3%, and 33% [11, 13, 14]. The increase in the prevalence of resistant strains, as a general trend, has been reported in recent years in several other studies [12, 15, 16]. There are also large regional differences in the occurrence and proportion of MDR strains [11, 14].

In our cohort, the most common strains were *Klebsiella spp.* (29%), *E. coli* (24%) and *P. aeruginosa* (13%). Most similar studies have reported that *E. coli* is the dominant uropathogen in SCI/D patients, with a significantly lower proportion of *Klebsiella spp.* [12, 14, 17]. The relatively high proportion of patients managed with UC/SC due to the acute nature of the spinal ward is a possible reason for the high incidence of infection with *Klebsiella spp.* in our group. Most patients are hospitalized in this ward for an average of three months after the injury before being transferred to a special rehabilitation institution for patients with SCI/D. In the cohort, 15% of patients with polytrauma were receiving long-term management with UC/SC. This may partially explain the high prevalence of MDR strains and the identification of nosocomial strains of *Klebsiella spp*. Another explanation may be the frequent use of broad-spectrum antibiotics for indications other than UTIs, which also leads to the selection of MDR strains.

We observed an increase in ESBL production in *Enterobacteriaceae* (26%), and the previous use of fluoroquinolones and third- and fourth-generation cephalosporins appears to be a risk factor for ESBL production [18]. The increasing trend in ESBL production was also confirmed in another study that identified ESBL production in 6.6% of *E. coli* and *K. pneumoniae* strains [19]. We did not observe carbapenem resistance in our cohort, and the estimated rate of CRE in SCI/D patients in other studies was 1.7-7.6% [13, 20]. Compared with other studies, this study reported a lower prevalence of MRSA [6, 21]. Thus, there is a clear trend, with a shift among MDR bacteria from gram-positive cocci to GNB [6].

Based on this overview of bacterial strains and the rates of resistance to various antibiotics, there is clear evidence of a high proportion of nosocomial strains, mostly GNB. Bacterial colonization occurs through

the spread of strains derived from the intestinal microflora, perineum, or urethra when the catheter is manipulated [22]. Contamination from the external environment around the patient and transmission between patients and by medical staff are also common. Colonization can persist in the long term without any signs of an acute UTI. However, if colonization occurs, the patient is at risk for lifelong recurrent UTIs. The prevalence of multidrug resistance and other types of resistance in the population varies between hospitals, individual wards and specific patient populations. The prevalence of resistance is influenced by the specific patient population, antibiotic policies and established clinical practices.

In our work, UC/SC bladder management, male sex, and injury severity were identified as risk factors for multidrug resistance. Other studies have reported comparable findings [19, 23–25]. The most common risk factor was management with UC/SC. Other risk factors included a history of UTIs, previous antimicrobial therapy, and prolonged and repeated exposure to antimicrobials [26]. One of the basic measures for the prevention of multidrug resistance should be the early removal of indwelling catheters, and the prophylactic effect of management with CIC has been shown [27–30]. Another risk factor was spontaneous voiding. We consider this to be evidence of long-term colonization that persisted after switching from UC/SC to spontaneous voiding.

The results of our work showed a high level of resistance to commonly used antibiotics, especially aminopenicillins, amoxicillin-clavulanic acid, cephalosporins, fluoroquinolones and SMX-TMP, which are commonly used to treat UTIs. Our results correspond to the findings in other studies [12, 25, 31].

Our work is limited by a number of factors. The first factor is that it was a retrospective collection of data from one center. Although the sample size was relatively large, the findings need to be validated in a multicenter study. Second, clinically symptomatic infections and asymptomatic bacteriuria were not considered separately. Third, the results of the study could have been affected by regional trends, established clinical practices, and local antibiotic policies.

Conclusion

In this large cohort of patients, we observed increasing resistance among uropathogens and a high prevalence of MDR strains in the inpatient SCI/D population. In particular, the use of an indwelling catheter is a risk factor for infection with MDR bacteria.

Abbreviations

AST- Antimicrobial susceptibility testing, CFU-Colony forming unit, CIC-Clean intermittent catheterization, CRE- Carbapenem resistance, ESBL- Extended-spectrum beta-lactamases, GNB - Gram-negative bacteria, MDR - Multidrug resistant, PDR- Pandrug resistant, SCI/D - Spinal cord injury and disorders, VRE – Vancomycin resistance, UTI - Urinary tract infection, XDR-Extensively drug resistant

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with Good Clinical Practices and the principles of the Declaration of Helsinki. The study was approved by the relevant Institutional Review Boards of Krajská nemocnice Liberec. Written informed consent was obtained from all study participants.

Consent for publication

All authors consent to the publication of the manuscript in BMC Infectious Diseases

Availability of data and materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interests.

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Authors' contributions

Study concept and desing: VŠ, VP. Data analysis and interpretation: VŠ, HK. Microbiology expertize: DF. Manuscript preparation: VŠ, VP. Manuscript review: VŠ, JM, JŠ. All authors read and approved the final manuscript .

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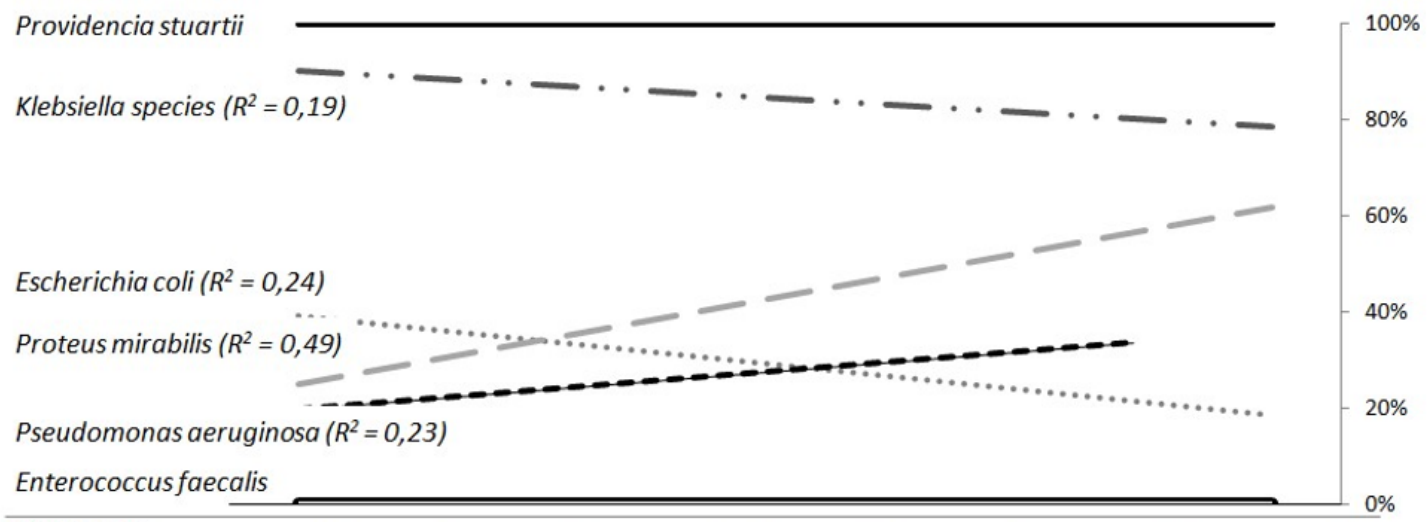
Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures



Bacterial strain N (%) of MDR | Total N *	2013	2014	2015	2016	2017	2018	2019	2020
Klebsiella species	42 (88%) | 48	55 (79%) | 70	57 (93%) | 61	56 (86%) | 65	44 (90%) | 49	49 (94%) | 52	33 (79%) | 42	10 (67%) | 15
Escherichia coli	23 (42%) | 55	22 (41%) | 54	13 (30%) | 44	8 (12%) | 66	8 (24%) | 34	15 (48%) | 31	8 (28%) | 29	1 (6%) | 16
Pseudomonas aeruginosa	2 (9%) | 23	10 (30%) | 33	9 (38%) | 24	5 (25%) | 20	6 (19%) | 31	8 (27%) | 30	7 (39%) | 18	1 (100%) | 1
Enterococcus faecalis	0 (0%) | 18	0 (0%) | 43	0 (0%) | 29	0 (0%) | 24	0 (0%) | 25	0 (0%) | 16	0 (0%) | 13	0 (0%) | 6
Proteus mirabilis	7 (39%) | 18	4 (29%) | 14	3 (20%) | 15	3 (25%) | 12	10 (50%) | 20	10 (67%) | 15	17 (68%) | 25	7 (50%) | 14
Providencia stuartii	13 (100%) | 13	7 (100%) | 7	6 (100%) | 6	5 (100%) | 5	7 (100%) | 7	5 (100%) | 5	1 (100%) | 1	7 (100%) | 7

** Total N = N of MDR + N of non MDR*

Figure 1

Proportion of MDR strains by study year

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.xlsx
- Table2.xlsx
- Table3.xlsx
- Table4Rev1.xlsx
- Table5.xlsx

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