Prevalence of antibiotic resistance among blood pathogens isolated from routine laboratory specimens at Livingstone Central Hospital in Zambia

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Research Article

Keywords: Antibiotic resistance, Blood stream infections, Imipenem, Escherichia coli Livingstone Central Hospital

Posted Date: May 26th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1695387/v1

Abstract

Introduction. Bloodstream infections (BSI) are a major public health burden with high mortality, and when coupled with antimicrobial resistance, the healthcare costs increase. This study aimed to establish the prevalence of antimicrobial resistance patterns of pathogens isolated from blood cultures at Livingstone Central Hospital (LCH) from 2019 to 2021.

Methods. A single-centre laboratory based retrospective study with information collected from electronic laboratory system generated reports on all isolated organisms at LCH microbiology laboratory for a period of 3 years.

Results. A total of 765 specimens were processed from January 2019 to December 2021 and only 331 (43.3%) met the inclusion criteria. More specimens from female (61.3%) than males (38.7%), and from out-patient departments (65.9%) than in-patient departments (34.1%) specimens were processed. Amongst the bacteria isolates identified, *Escherichia coli* (27.2%) was the commonest isolate followed by *Enterobacter agglomerans* (22.7%), *Klebsiella pneumoniae* (13%), *Klebsiella oxytoca* (6.3%), *Enterobacter aerogenes* (5.4%), *Enterobacter cloacae* (5.4%), *Citrobacter freundii* (C4.8%), *Serratia marcescens* (3.6%), *Proteus mirabilis* (3.3%), and *Staphylococcus aureus* (2.7%). Antibiotic susceptibility testing identified the least potent antibiotic as ampicillin (92.9%) followed by co-trimoxazole (82.7%), nalidixic acid (68.3%), penicillin (66.7%), tetracycline (63.5%), and chloramphenicol (50.3%) whereas the most effective antibiotic was imipenem (84.6%) followed by norfloxacin (64.7%) and nitrofurantoin (61.2%). Resistance maybe affected by patient gender and location.

Conclusion. Multidrug resistance strains causing BSI are increasing and imipenem is still effective but risk being over-used. This will impact healthcare costs and increase mortality rates at the hospital level.

Introduction

Bloodstream infections (BSI) are infectious diseases recognized by the presence of viable microorganisms in the bloodstream [1]. Globally, BSI are a major public health burden with high mortality as the infections account for 10–20% of all nosocomial infections and are the eighth leading cause of mortality (15%) in the United States [2–4]. In BSI, patient outcome is critically influenced by delayed therapy, and fast and accurate pathogen diagnostics decisively improves the care of patients [4]. In spite of the increased mortality rate that accompanies BSI, prolong patient stay in the hospital and increased health care costs had been reported, and inadequate empirical therapy is usually associated with adverse outcomes [2]. The mechanism of quick identification of pathogens (like automated blood culture systems) and their susceptibility patterns in patients with bacteraemia is lacking in many healthcare facilities. Few healthcare facilities especially those in urban areas use the traditional method of blood culture bottles that are usually incubated for a number days (before declaring them negative) with daily subcultures on solid media that subsequently increase the chances of false positives due to possible contamination [5]. Because of this, broad spectrum antibiotics are liberally and mostly unnecessarily

used resulting to an increase in emerging resistance and when combined with poor infection control practices, resistant bacteria can easily be disseminated to other patients and the environment [6].

Antimicrobial resistance (AMR) is another major public health concern, worldwide. In low and medium-income countries (LMIC), AMR monitoring is inadequate, but the extensive usage of antibiotics to prevent and treat infectious diseases has led to the emergence and spread of antibiotic resistance which has influenced a particular force on susceptible bacteria leading to resistant strain survival, consequently increasing medical costs, illness, and mortality [8]. Therefore, surveillance of bloodstream infections from blood cultures and their antibiotic resistance patterns are vital to the care of patients and prevention of BSI [2]. Studies have shown increased prevalence of extended spectrum beta lactamase (ESBL) producing strains and carbapenem resistance strains among blood pathogens [7–9].

In Zambia, several reports have indicated the prevalence of antibiotic resistant bacterial pathogens [10–14], and more recently, a spike in resistance to imipenem, ciprofloxacin and ampicillin against *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* and *Proteus* species from blood and other specimen sources was observed [15]. Despite these reports, irrational use of antibiotics is widely practiced and lack of control policies on over-the-counter antibiotics increase emergence of multidrug resistant (MDR) strains. To circumvent the MDR issue at a hospital level, monitoring of the pathogenic spectrum and changes in bacterial antibiotic resistance help in effective clinical therapy and infection control [16]. Therefore, our study aimed at understanding the resistance patterns of pathogens isolated from blood cultures at Livingstone Central Hospital (LCH).

Material And Methods

Study design and site. A hospital based retrospective study was conducted at Livingstone Central Hospital (LCH) on routine specimen isolates from patients who visited the hospital between January 2019 – December 2021. The cultures of patient specimens, identification of bacterial isolates and antimicrobial susceptibility testing were conducted on commercially and in-house prepared media, but quality controlled with American Type Culture Collection (ATCC) standard strains following the Clinical and Laboratory Standards Institute (CLSI) recommendations [17]. The LCH microbiology laboratory participates in a bacteriology External Quality Assessment (EQA) program and has been accredited by the Southern African Development Community Accreditation Service (SADCAS).

Eligibility criteria. This study included all specimens having information on the patient's gender, age, location (ward/clinic), name of organism and antibiotic susceptibility testing. However, any isolated organism without a species name, and with unknown source (i.e., lack of age-, location-, and gender of patient, and sample type) were excluded from the study.

Data collection and analysis. Data from electronic laboratory system generated reports on all isolated organisms at LCH microbiology laboratory for a period of 3 years (January 2019 to December 2021) was used from which information such as age, gender, patient's location, name of the organism and the antibiotic susceptibility were considered. The collected data was entered, assorted, and coded using

Microsoft Excel 365 and then exported to IBM Statistical Package for Social Science (SPSS) version 20 for analysis. Descriptive statistics was used to describe our data. Microsoft Excel 365 was used for graph generation. A chi-square test was used for categorical variables. A p-value of ≤ 0.05 was considered statistically significant.

Ethical consideration. The study considered data from the hospital laboratory generated reported for routine laboratory diagnosis and was granted ethical waiver from Mulungushi University School of Medicine and Health Sciences Research Ethics Committee (reference no.: SMHS-MU2-2021-33v1) while permission to use the Disa*Lab system generated data was obtained from Livingstone Central Hospital management. No personal identifiers were included in the study.

Results

Characterization of bacterial organisms isolated from blood specimens

A total of 765 specimens were processed from January 2019 to December 2021 and only 331 (43.3%) met the inclusion criteria for this study. Of the 331, 203(61.3%) specimens were received from female and 128 (38.7%) from males, ranging age between 0 and 80 years with the median age of 32 ± 20.9 years. Categorizing the age groups in 0–16 years, 17–39 years, and 40–80 years, 55 (16.6%)-, 171 (51.7%)-, and 105 (31.7%)- specimens were received, respectively (Fig. 1). Most specimens came from out-patient departments (OPD; 218, 65.9%) while 113 (34.1%) came from the in-patient departments (IPD), and 104 (31.4%) specimens were analysed in the year 2019, while 130 (39.1%) for the year 2020 and 97 (29.3%) were processed in year 2021 (Fig. 1). Amongst the bacteria isolates identified, *Escherichia coli* (*E. coli*; 90, 27.2%) was the commonest isolate followed by *Enterobacter agglomerans* (*E. agglomerans*; 75, 22.7%), *Klebsiella pneumoniae* (*K. pneumoniae*; 43, 13%), *Klebsiella oxytoca* (*K. oxytoca*; 21, 6.3%), *Enterobacter aerogenes* (*E. aerogenes*; 19, 5.4%), *Enterobacter cloacae* (*E. cloacae*; 18, 5.4%), *Citrobacter freundii* (*C. freundii*; 16, 4.8%), *Serratia marcescens* (*S. marcescens*; 12, 3.6%), *Proteus mirabilis* (*P. mirabilis*; 11, 3.3%), and *Staphylococcus aureus* (*S. aureus*; 9, 2.7%) as presented in Fig. 2.

Antibiotic susceptibility testing was conducted with a panel of antibiotics that are commonly used at LCH. The resistance percentage of used antibiotics showed ampicillin (92.9%) as the least effective drug followed by co-trimoxazole (82.7%), nalidixic acid (68.3%), penicillin (66.7%), tetracycline (63.5%), and chloramphenicol (50.3%) whereas the most effective antibiotics were imipenem (15.4%), norfloxacin (35.3%) and nitrofurantoin (38.8%) as shown in Fig. 3.

In trying to understand the association of independent variables with antibiotic resistance, we conducted a chi-square test. According to this analysis, the resistance patterns of *E. agglomerans* to nitrofurantoin ($p = 0.041$), *E. coli* to cefuroxime ($p = 0.014$), *K. oxytoca* to cefuroxime ($p = 0.026$) were affected by patient gender (Table 1). Furthermore, patient location (IPD vs OPD) had a negative effect on chloramphenicol and ciprofloxacin. *E. agglomerans* and *K. pneumoniae* isolates from IPD were resistant to

chloramphenicol (p = 0.002) and ciprofloxacin (p = 0.021) whereas the resistance of *E. aerogenes* to co-trimoxazole (p = 0.008) and *E. coli* to ampicillin (p = 0.034) varied on the year the bacteria were isolated (Table 1).

Table 1
The resistant pattern of some blood isolated bacteria with respect to patient gender, patient location, and year of isolation.

Gender					
Microorganism	**Female**	**Male**		**p-value**	**Drug**
E. agglomerans	30.3% (10/33)	66.7% (8/12)		0.041	Nitrofurantoin
E. coli	10% (1/10)	100% (3/3)		0.014	Cefuroxime
K. oxytoca	0% (0/7)	62.5% (5/8)		0.026	Cefotaxime
Location					
Microorganism	**IPD**	**OPD**		**p-value**	**Drug**
E. agglomerans	87.5% (7/8)	15.4% (2/13)		0.002	Chloramphenicol
K. pneumoniae	100% (6/6)	28.6% (2/7)		0.021	Ciprofloxacin
Year					
Microorganism	**2019**	**2020**	**2021**	**p-value**	**Drug**
E. aerogenes	100% (10/10)	40% (2/5)	0% (0/1)	0.008	Co-trimoxazole
E. coli	83.3% (5/6)	95.2% (40/42)	74.1% (20/27)	0.034	Ampicillin

Discussion

Bacteraemia is the presence of viable bacteria in the circulatory system that may result from an existing focus of infection, a site with commensal flora or direct inoculation of contaminants via a trauma. Our study found that bacteraemia cases at LCH was mostly from female patients (61.3%) and were community-acquired as the OPD (65.9%) recorded the highest numbers of infections. Furthermore, increasing in age was a risk factor for BSI because the age groups 17-39years (51.7%) and 40-80years (31.7%) had more BSI than the paediatric age group (0-16years). This agreed with Laupland et al. [18] study that showed older and male patients of having increased risk of contracting BSI but disagreed on the at-risk gender as our study indicated more BSI from the female patients. This could be due to the variations in the types of studies and the study population as they. The findings of more community-acquired BSI agreed with another retrospective study conducted in the United Stated by Page *et al*. study [19] where more cases of community-acquired severe sepsis than healthcare-associated severe sepsis and hospital-acquired severe sepsis.

The commonest blood pathogens at LCH were *E. coli* (27.2%), *E. agglomerans* (22.7%), *K. pneumoniae* (13%), *K. oxytoca* (6.3%), *E. aerogenes* (5.4%), *E. cloacae* (5.4%), *C. freundii* (4.8%), *S. marcescens* (3.6%), *P. mirabilis* (3.3%), and *S. aureus* (2.7%). Similarly, Enterobacteriaceae were the most prevalent microorganisms followed by Coagulase-negative *Staphylococci* and *S. aureus* in people living with HIV [20] and another study [21] that found *E. coli* as the most common cause of bacteraemia.

The highest prevalence of resistant strains was discovered in this study. Ampicillin lost its potency by 92.9% followed by co-trimoxazole (82.7%), nalidixic acid (68.3%), penicillin (66.7%), and tetracycline (63.5%). Studies have reported an increased trend of BSI caused by methicillin-resistant *S. aureus* (MRSA) and third-generation cephalosporin-resistant *E. coli* which subsequently exert pressure on prolonged hospital stay thereby increasing the healthcare costs [22]. Our study revealed high numbers of gram-negative bacteria that showed resistance to a wide range of antibiotics including ceftazidime (42.9%), cefuroxime (42.9%) and cefotaxime (44.6%). This is an indication that whenever, a gram-negative bacterium is identified in BSI, careful selection of therapeutic drugs should be evidence-based because the practice of commencing patients on broad-spectrum antibiotics has contributed to the multidrug resistance development being observed today. Furthermore, our study found imipenem with the sensitivity of 84.6%, norfloxacin with 64.7% and nitrofurantoin with 61.2%. This finding was in concordance with Serretiello *et al*. study [23] that found *E. coli* with high sensitivity to carbapenems and amikacin. Despite these antibiotics retaining their potencies against the isolated blood pathogens, the pathogens were slowing exhibiting resistances perhaps due to their over-use, and hence implementing effective antimicrobial resistance surveillance system such as antimicrobial stewardship programs and/or using the global antimicrobial resistance surveillance system(GLASS) should be considered by various hospitals [21].

In addition, our study found *E. agglomerans* resistance to nitrofurantoin ($p = 0.041$), *E. coli* resistance to cefuroxime ($p = 0.014$), and *K. oxytoca* resistance to cefuroxime ($p = 0.026$) were high in male patients by 66.7%, 100% and 62.5%, respectively. Additionally, *E. agglomerans* resistance to chloramphenicol ($p = 0.002$) and *K. pneumoniae* resistance to ciprofloxacin ($p = 0.021$) were high in IPD by 87.5% and 100%, respectively. Through unclear mechanisms, males are more prone to having MDR-BSI as discovered in this study and supported by other studies [24–26]. Moreover, there is an increased chance of having MDR BSI amongst hospitalized patients due to the over-use of assorted chemotherapy [19, 27].

Multidrug-resistant (MDR) pathogens are making common infections more difficult to treat or untreatable, and burdensome to the health care system. Studies have shown increased prevalence of ESBL producing strains and carbapenem resistance strains among blood pathogens [7–9]. The MDR patterns of bacterial isolates from our study clearly suggest that ESBL producing strains may be circulating at LCH. Intensifying infections control and prevention practices, periodic antibiogram studies and implementing the antimicrobial stewardship program may reduce the generation of MDR strains and regulate antibiotic prescribing at the hospital level.

Conclusion

Our study had shown a rise in MDR strains causing blood stream infections. Treating these infections, imipenem was still effective but risk being over-used. Furthermore, Enterobacteriaceae and *S. aureus* were the commonest cause of blood stream infections at Livingstone Central Hospital. Therefore, the choice of antibiotics to manage Enterobacteriaceae- and *S. aureus*-related blood stream infections should be evidenced-based to reduce the MDR strains to a minimum, and the prescribing of broad-spectrum antibiotics should be condemned or considered as a last resort. Otherwise, this alarming antibiotic picture may trickle down to the high cost of managing bacterial infections consequently increasing mortality and morbidity rates.

Declarations

Acknowledgment

We would like to sincerely thank all members of staff in the Laboratory department at Livingstone Central Hospital for their work in generating data used in this study.

Conflict of Interest

All authors declared no conflict of interest

Contributions

TNM did data collection, analysis and wrote the manuscript. WC conceptualized the study, performed data analysis, and wrote the manuscript. All authors approved the current version of the manuscript.

References

1. C. Viscoli, Bloodstream Infections: The peak of the iceberg, Virulence 7(3) (2016) 248–251.
2. P. Kp, V. Arora, G. Pp, Bloodstream Bacterial Pathogens and their Antibiotic Resistance Pattern in Dhahira Region, Oman, Oman Med J 26(4) (2011) 240–279.
3. R.P. Wenzel, M.B. Edmond, The impact of hospital-acquired bloodstream infections, Emerging infectious diseases 7(2) (2001) 174.
4. B. Lamy, M. Sundqvist, E.A. Idelevich, Bloodstream infections - Standard and progress in pathogen diagnostics, Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases 26(2) (2020) 142–150.
5. M.L. Towns, W.R. Jarvis, P.R. Hsueh, Guidelines on blood cultures, J Microbiol Immunol Infect 43(4) (2010) 347–9.
6. M. Akova, Epidemiology of antimicrobial resistance in bloodstream infections, Virulence 7(3) (2016) 252–266.
7. D.K. Saeed, J. Farooqi, S. Shakoor, R. Hasan, Antimicrobial resistance among GLASS priority pathogens from Pakistan: 2006–2018, BMC infectious diseases 21(1) (2021) 1231–1231.

8. J.A. Opintan, M.J. Newman, Prevalence of antimicrobial resistant pathogens from blood cultures: results from a laboratory based nationwide surveillance in Ghana, Antimicrobial Resistance & Infection Control 6(1) (2017) 64.
9. S. Gandra, N. Mojica, E.Y. Klein, A. Ashok, V. Nerurkar, M. Kumari, U. Ramesh, S. Dey, V. Vadwai, B.R. Das, R. Laxminarayan, Trends in antibiotic resistance among major bacterial pathogens isolated from blood cultures tested at a large private laboratory network in India, 2008–2014, International journal of infectious diseases: IJID : official publication of the International Society for Infectious Diseases 50 (2016) 75–82.
10. T. Mulongo, K. Kamvuma, C.N. Phiri, J.A. Mulemena, W. Chanda, Elevators and staircase handrails as potential sources of nosocomial pathogens at Ndola Teaching Hospital, Zambia, (2021).
11. W. Chanda, J.A. Mulemena, M. Manyepa, K. Kamvuma, Antimicrobial stewardship and oxazolidinones use, a consideration for a Zambian health system, Medical Journal of Zambia 46(3) (2019) 165–171.
12. W. Chanda, M. Manyepa, E. Chikwanda, V. Daka, J. Chileshe, M. Tembo, J. Kasongo, A. Chipipa, R. Handema, J.A. Mulemena, Evaluation of antibiotic susceptibility patterns of pathogens isolated from routine laboratory specimens at Ndola Teaching Hospital: A retrospective study, PLOS ONE 14(12) (2019) e0226676.
13. H. Chiyangi, J.B. Muma, S. Malama, J. Manyahi, A. Abade, G. Kwenda, M.I. Matee, Identification and antimicrobial resistance patterns of bacterial enteropathogens from children aged 0–59 months at the University Teaching Hospital, Lusaka, Zambia: a prospective cross sectional study, BMC infectious diseases 17(1) (2017) 1–9.
14. S.E. Mshana, M. Matee, M. Rweyemamu, Antimicrobial resistance in human and animal pathogens in Zambia, Democratic Republic of Congo, Mozambique and Tanzania: an urgent need of a sustainable surveillance system, Annals of clinical microbiology and antimicrobials 12(1) (2013) 1–10.
15. M. Kasanga, R. Mukosha, M. Kasanga, M. Siyanga, S. Mudenda, B.B. Solochi, M. Chileshe, M.J. Mwiikisa, T. Gondwe, T. Kantenga, A.L. Shibemba, R. Nakazwe, M. Chitalu, J. Wu, Antimicrobial resistance patterns of bacterial pathogens their distribution in university teaching hospitals in Zambia, Future microbiology 16 (2021) 811–824.
16. L. Tian, Z. Zhang, Z. Sun, Antimicrobial resistance trends in bloodstream infections at a large teaching hospital in China: a 20-year surveillance study (1998–2017), Antimicrobial Resistance & Infection Control 8(1) (2019) 86.
17. CSLI, Performance Standards for antimicrobial susceptibility testing, 26 ed., Clinical and laboratory standards institute, Wayne PA, 2016.
18. K.B. Laupland, K. Pasquill, L. Steele, E.C. Parfitt, Burden of bloodstream infection in older persons: a population-based study, BMC Geriatrics 21(1) (2021) 31.
19. D.B. Page, J.P. Donnelly, H.E. Wang, Community-, Healthcare-, and Hospital-Acquired Severe Sepsis Hospitalizations in the University HealthSystem Consortium, Crit Care Med 43(9) (2015) 1945–51.

20. E. Franceschini, A. Santoro, M. Menozzi, E. Bacca, C. Venturelli, S. Zona, A. Bedini, M. Digaetano, C. Puzzolante, M. Meschiari, G. Cuomo, G. Orlando, M. Sarti, G. Guaraldi, A. Cozzi-Lepri, C. Mussini, Epidemiology and Outcomes of Bloodstream Infections in HIV-Patients during a 13-Year Period, Microorganisms 8(8) (2020).
21. R. Sirijatuphat, K. Sripanidkulchai, A. Boonyasiri, P. Rattanaumpawan, O. Supapueng, P. Kiratisin, V. Thamlikitkul, Implementation of global antimicrobial resistance surveillance system (GLASS) in patients with bacteremia, PLOS ONE 13(1) (2018) e0190132.
22. M.E.A. de Kraker, P.G. Davey, H. Grundmann, B.s.g. on behalf of the, Mortality and Hospital Stay Associated with Resistant Staphylococcus aureus and Escherichia coli Bacteremia: Estimating the Burden of Antibiotic Resistance in Europe, PLOS Medicine 8(10) (2011) e1001104.
23. E. Serretiello, B. Santella, V. Folliero, D. Iervolino, E. Santoro, R. Manente, F. Dell'Annunziata, R. Sperlongano, V. Crudele, A. De Filippis, M. Galdiero, G. Franci, G. Boccia, Prevalence and Antibiotic Resistance Profile of Bacterial Pathogens in Aerobic Vaginitis: A Retrospective Study in Italy, Antibiotics (Basel) 10(9) (2021) 1133.
24. F. Jauréguy, E. Carbonnelle, S. Bonacorsi, C. Clec'h, P. Casassus, E. Bingen, B. Picard, X. Nassif, O. Lortholary, Host and bacterial determinants of initial severity and outcome of Escherichia coli sepsis, Clinical Microbiology and Infection 13(9) (2007) 854–862.
25. V. Karamouzos, E.J. Giamarellos-Bourboulis, D. Velissaris, T. Gkavogianni, C. Gogos, Cytokine production and outcome in MDR versus non-MDR gram-negative bacteraemia and sepsis, Infectious Diseases 53(10) (2021) 764–771.
26. Y.-C. Lee, C.-Y. Hsiao, M.-C. Hung, S.-C. Hung, H.-P. Wang, Y.-J. Huang, J.-T. Wang, Bacteremic Urinary Tract Infection Caused by Multidrug-Resistant Enterobacteriaceae Are Associated With Severe Sepsis at Admission: Implication for Empirical Therapy, Medicine (Baltimore) 95(20) (2016) e3694-e3694.
27. A. Despotovic, B. Milosevic, I. Milosevic, N. Mitrovic, A. Cirkovic, S. Jovanovic, G. Stevanovic, Hospital-acquired infections in the adult intensive care unit—Epidemiology, antimicrobial resistance patterns, and risk factors for acquisition and mortality, American journal of infection control 48(10) (2020) 1211–1215.

Figures

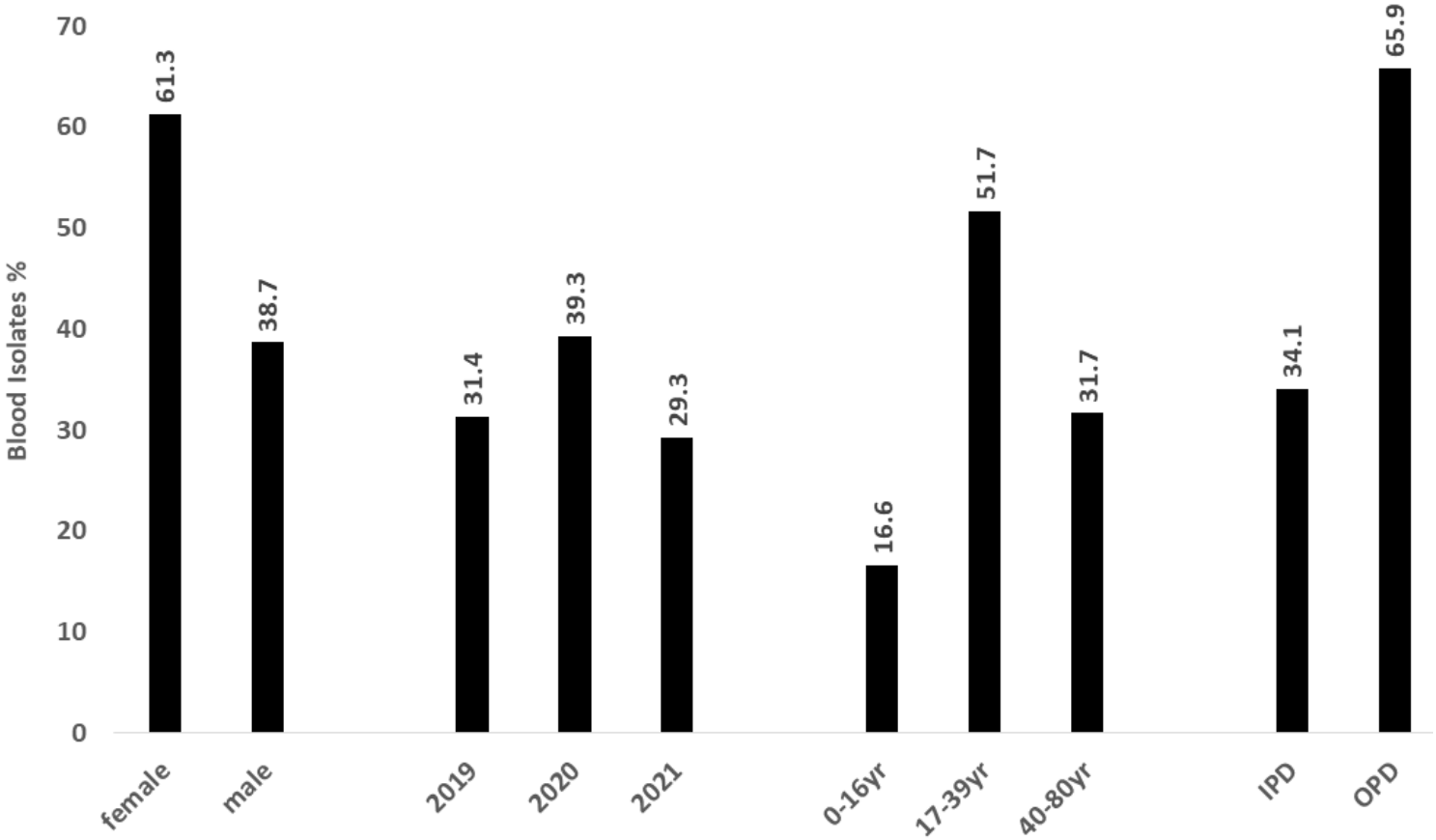

Figure 1

Percentage frequencies of blood isolates based on patient gender, year of isolation, patient age group and patient location. IPD: in-patient departments, OPD: out-patient departments, yr: year.

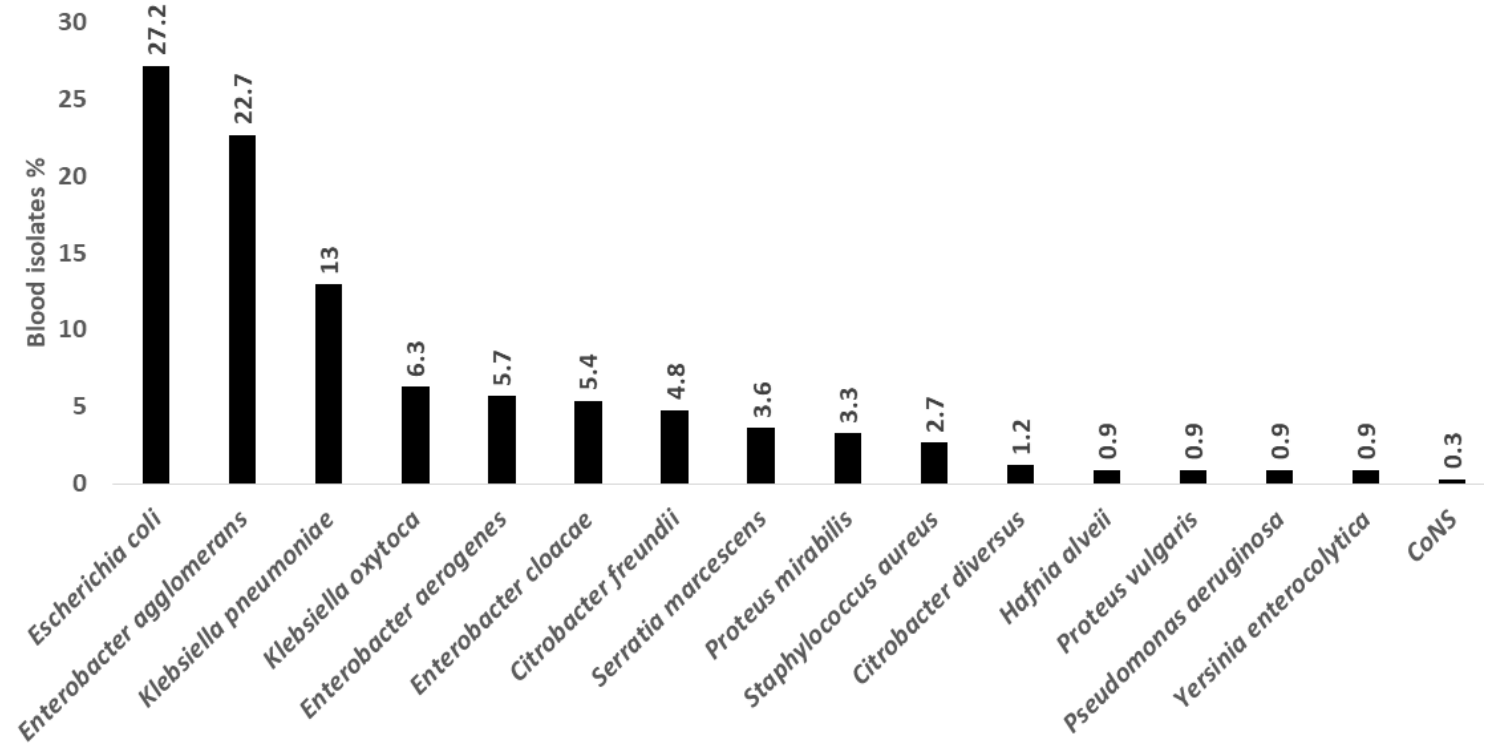

Figure 2

Percentage frequencies of the type of bacterial isolates from blood specimens. CoNS: Coagulase negative *Staphylococci*.

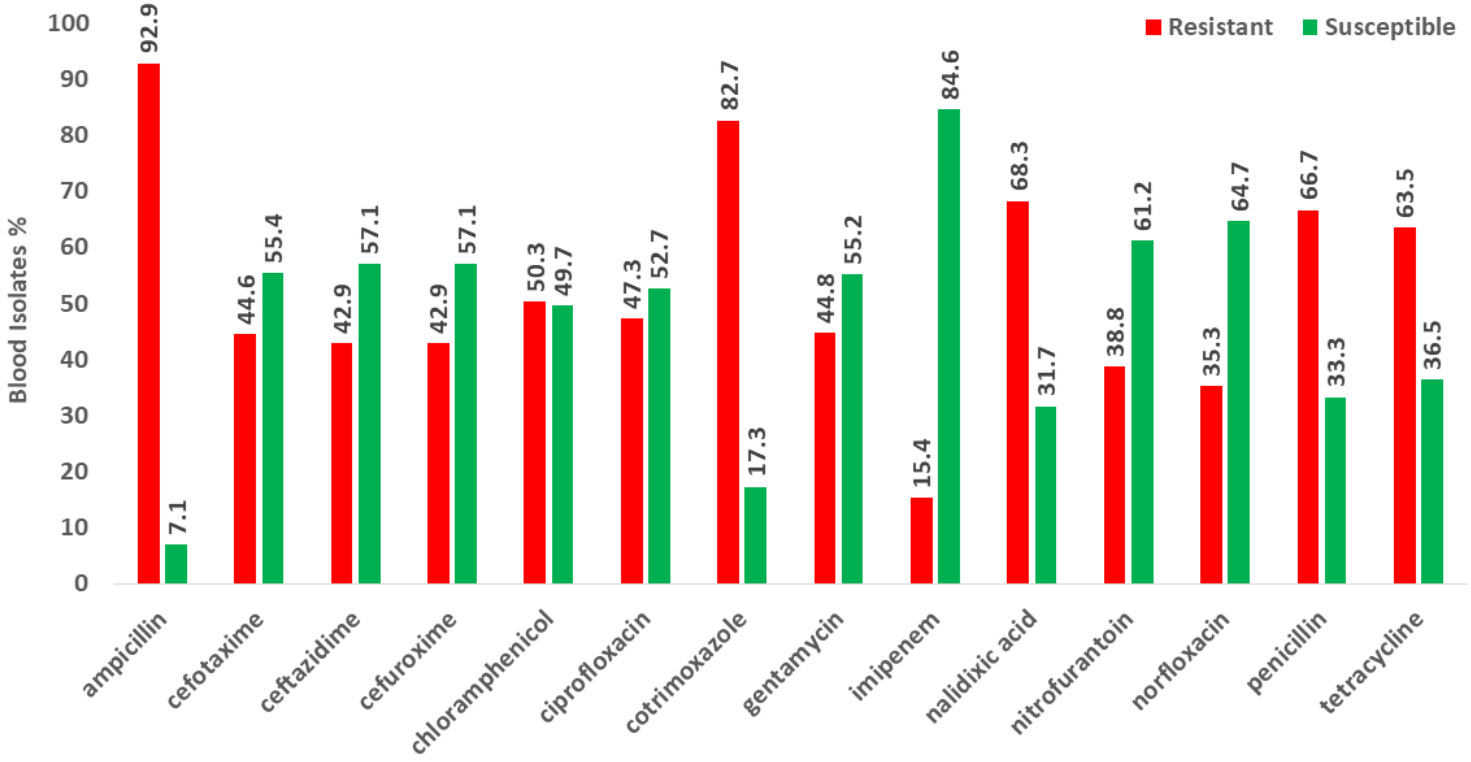

Figure 3

Percentage frequencies of the susceptibility pattern of blood isolates.