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RESEARCH ARTICLE

Antibiotic susceptibility patterns of pathogens isolated from laboratory specimens at Livingstone Central Hospital in Zambia

Thresa N. Mwansa, Kingsley Kamvuma, John Amos Mulemena, Christopher Newton Phiri, Warren Chanda*

Department of Pathology and Microbiology, Mulungushi University School of Medicine and Health Sciences, Livingstone, Zambia

* chandawarren@yahoo.com

Abstract

Background

Multidrug resistance (MDR) is a global problem that require multifaceted effort to curb it. This study aimed to evaluate the antibiotic susceptibility patterns of routinely isolated bacteria at Livingstone Central Hospital (LCH).

Methods

A retrospective study was performed on all isolated organisms from patient specimens that were processed from January 2019 to December 2021. Specimens were cultured on standard media and Kirby-Bauer disc diffusion method was employed for susceptibility testing following the Clinical and Laboratory Standard Institute's recommendations.

Results

A total of 765 specimens were processed and only 500 (65.4%) met the inclusion criteria. Of the 500, 291(58.2%) specimens were received from female and from the age-group 17–39 years (253, 50.6%) and 40–80 years (145, 29%) in form of blood (331, 66.2%), urine (165, 33%) and sputum (4, 0.8%). Amongst the bacterial isolates, *Staphylococcus aureus* (142, 28.4%) was the commonest followed by *Escherichia coli* (91, 18.2%), and *Enterobacter agglomerans* (76, 15.2%), and *Klebsiella pneumoniae* (43, 8.6%). The resistance pattern revealed ampicillin (93%) as the least effective drug followed by oxacillin (88%), penicillin (85.6%), co-trimoxazole (81.5%), erythromycin (71.9%), nalidixic acid (68%), and ceftazidime (60%) whereas the most effective antibiotics were imipenem (14.5%), and piperacillin/tazobactam (16.7%). The screening of methicillin resistant *Staphylococcus aureus* (MRSA) with cefoxitin showed 23.7% (9/38) resistance.

Conclusion

Increased levels of MDR strains and rising numbers of MRSA strains were detected. Therefore, re-establishing of the empiric therapy is needed for proper patient management,

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studies to determine the levels of extended spectrum beta lactamase- and carbapenemase-producing bacteria are warranted.

Introduction

Antibiotics are essential commodities in managing bacterial infections in humans and animals. However, the spread of antibiotic-resistant bacteria had been increased by the overuse and misuse of antibiotics as well as social and economic factors [1]. Therefore, antibiotic resistance is a public health concern that has been recognized globally. Studies have indicated that a lot of lives are lost due to antimicrobial resistance related illnesses every year, and almost 100% β-lactams and some 3rd generation cephalosporins and carbapenems have recorded resistance by some organisms [2].

Prior to the development of the first β-lactam (penicillin) and its release in medical practice, emergence of resistance had been reported and the first β-lactamase enzyme was identified in *Escherichia coli*(*E.coli*) [3]. Extended Spectrum β-lactamases (ESBLs) are enzymes that deactivate a variety of β-lactam antibiotics including penicillins, cephalosporins (3rd and 4th generations) and monobactams but less likely to deactivate cephamycins like cefoxitin [3–5]. Furthermore, the production of carbapenemase enzymes by some Enterobacteriaceae is alarming as these enzymes can break down antibiotics including carbapenem antibiotics, which are typically reserved to treat multidrug-resistant bacterial infections [6]. Carbapenem-resistant Enterobacteriaceae (CRE) causes very hard to treat infections because of being resistant to carbapenems that are used on multidrug resistant strains [7]. It is sufficed to note that the more bacteria get exposed to antimicrobial agents the more resistance develops. Therefore, there is need to preserve the current antimicrobial agents by using them judiciously otherwise, we risk having pandemic AMR infections that will devastate the global community.

In Africa, especially in low and medium-income countries (LMIC), antimicrobial resistance 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, illnesses and deaths of patients [8]. Therefore, antibiogram studies in LMIC are important to closely monitor the trends of AMR at hospital and national levels. This retrospective study aimed to assess antibiotics resistance among commonly isolated bacteria from routine specimens at Livingstone Central Hospital from 2019 to 2021.

Material and methods

Study design and site

A retrospective cohort study was conducted at Livingstone Central Hospital (LCH) on routine specimen isolates from paediatric and adults who visited the hospital between January 2019 – December 2021. Livingstone Central Hospital has different departments that include laboratory, internal medicine, surgery, paediatrics, and obstetrics and gynaecology. 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.

Bacterial culture and Identification

The bacteriological analysis involves culturing of specimen on appropriate quality controlled culturing media following the national standard operating procedures, and Clinical and Laboratory Standards Institutes (CLSI) guidelines [9]. All routine specimens from inpatients and outpatients were cultured on MacConkey, blood, and chocolate agar plates and incubated at 37˚C in an appropriate condition. Where applicable selective media such as mannitol salt agar and xylose lysine deoxycholate agar were used. Identification of bacterial isolates was aided with a Gram staining method, culture characteristics, motility test, and various biochemical tests such as oxidase test, triple sugar iron (TSI), indole production test, urease production test and citrate utilization test as described previously [9–11].

Antimicrobial susceptibility profile

The antibiotic susceptibility testing (AST) was performed using a Kirby-Bauer disc diffusion method on identified isolates by preparing the bacterial suspension in comparison with 0.5MacFarland turbidity standard and inoculating on Mueller-Hinton agar, and the choice of antibiotic discs selected as per CLSI guidelines [9] comprised ampicillin (10μg), cefotaxime (30μg), cefoxitin (30μg), ceftazidime(30μg), cefuroxime (30μg), cephalothin(30μg), chloramphenicol(30μg), ciprofloxacin(5μg), clindamycin(2μg), co-trimoxazole (1.25/23.73μg), doxycycline(30μg), erythromycin(15μg), gentamicin(10μg), imipenem (10μg), nalidixic acid (30μg), nitrofurantoin (300μg), norfloxacin (10μg), oxacillin (1μg), penicillin(10units), piperacillin (100μg), piperacillin/tazobactam (100/10μg), tetracycline (30μg), and vancomycin(30μg). The zones of inhibition were measured (in millimeters) and interpreted as susceptible and resistant (intermediate and resistant measurements) following the CLSI guidelines [9]. In addition, all penicillin-resistant *Staphylococcus aureus* (*S. aureus*) strains were screened with cefoxitin to detect methicillin resistant *S. aureus* (MRSA).

Quality control (QC)

Escherichia coli ATCC 25922 and *S. aureus* ATCC 25923 were used as QC for all antibiotics and culture media.

Data collection and analysis

Data from electronic laboratory system generated reports on all isolated organisms at LCH microbiology laboratory for three 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 2019 and then exported to Statistical Package for Social Science (SPSS) version 20 for analysis. Descriptive statistics was used to describe our data. Microsoft Excel 2019 and Graph prism 5 were used for graph generation. A chi-square test was used for categorical variables and a p-value of ≤ 0.05 was considered statistically significant.

Ethical consideration

This is a retrospective study that analysed secondary data generated from routine laboratory specimens from both adult and paediatric departments. There was no human interaction, and no personal identifiers were included in this study. However, ethical waiver was granted by Mulungushi University School of Medicine and Health Sciences Research Ethics Committee (SMHS-MU2-2021-33v1) while permission to use the Disa*Lab system generated data was obtained from Livingstone Central Hospital management.

Results

General characterization of isolated bacterial organisms

A total of 765 specimens were processed from January 2019 to December 2021 and only 500 (65.4%) met the inclusion criteria for this study. Of the 500, 291(58.2%) specimens were received from female and 209 (41.8%) from males, ranging age between 0 and 80 years with the mean average of 32.3±20.6 years. Categorizing the age groups in 0–16 years, 17–39 years, and 40–80 years, 102 (20.4%)-, 253 (50.6%)-, and 145 (29%)- specimens were received, respectively (Fig 1A). For the type of specimens requested, blood specimens were 331 (66.2%) followed by urine specimen (165, 33%) and sputum (4, 0.8%; Fig 1A). Most specimens came from out-patient department (OPD 323, 64.6%) while 177 (35.4%) came from the in-patient department (IPD), and 175 (35%) specimens were analysed in the year 2019 and 2020, while 150 (30%) specimens were processed in year 2021 (Fig 1A). Amongst the common bacterial isolates identified, *S. aureus* (142, 28.4%) was the commonest isolate followed by *E. coli* (91, 18.2%), *Enterobacter agglomerans* (*E. agglomerans*, 76, 15.2%), *Klebsiella pneumoniae* (*K. pneumoniae*, 43, 8.6%), and the least isolated bacteria were *Hafnia alvei*, *Proteus vulgaris* (*P. vulgaris*), and *Yersinia enterocolytica* (*Y. enterocolytica*, 3, 0.6% apiece; Fig 1C).

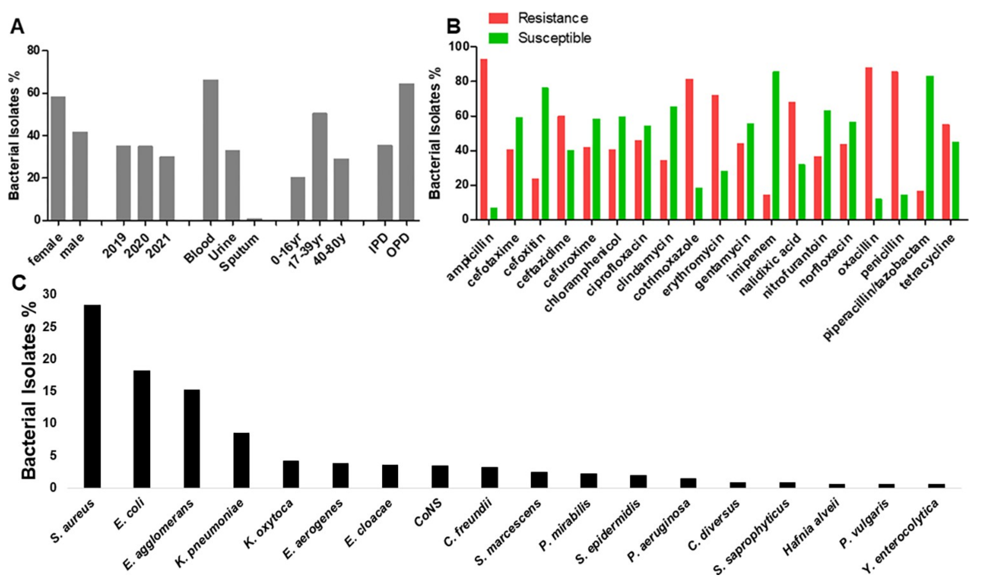

Fig 1. Characterization of bacterial isolates from blood, urine, and sputum specimens. (A) Bacterial isolates in percentages based on patient gender, year of isolated, sample type age and location; Majority specimens were blood and urine received from female patients, and from the OPD. (B) The susceptibility patterns (in percentages) against common utilized antibiotics. Multidrug resistance is presented with imipenem showing effectiveness. (C) Bacterial isolate from blood, urine, and sputum specimens in percentages. The graph shows *S. aureus* as a common isolate and *Y. enterocolytica* as least isolate at LCH. CoNS: Coagulase negative Staphylococci, IPD: in-patient department, OPD: out-patient department, yr/y: year.

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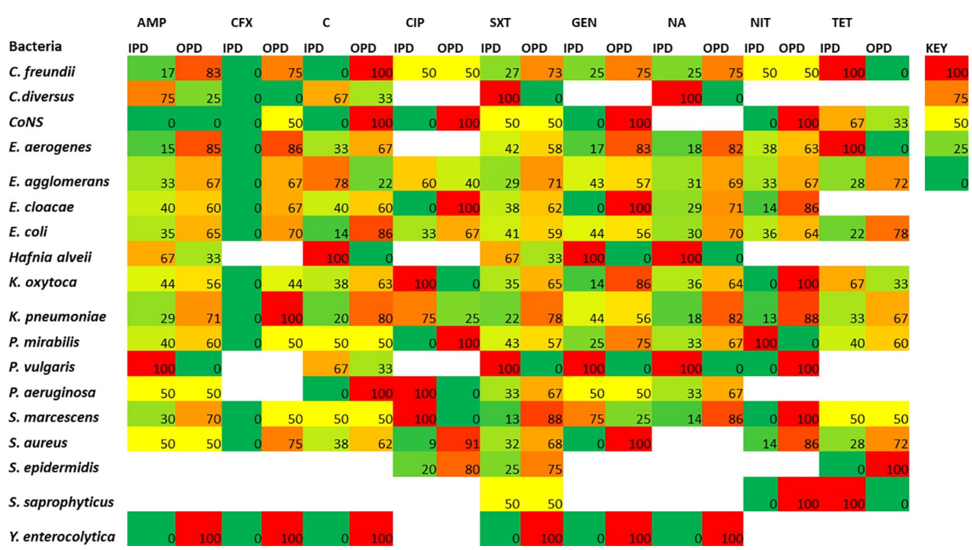
A large panel of antibiotics that are commonly used at LCH were used for antimicrobial susceptibility testing following the CLSI recommendations [9]. The resistance pattern indicated that ampicillin (93%) was the least effective drug followed by oxacillin (88%), penicillin (85.6%), co-trimoxazole (81.5%), erythromycin (71.9%), nalidixic acid (68%), ceftazidime (60%), tetracycline (55.1%), and ciprofloxacin (45.9%) whereas the most effective antibiotics were imipenem (14.5%), piperacillin/tazobactam (16.7%) and clindamycin (34.5%) as shown in Fig 1B. However, the screening of MRSA with cefoxitin showed 76.3% (29/38) susceptibility and 23.7% (9/38) resistance (Fig 1B). Furthermore, the antibiotic resistance distribution of bacteria from IPD and OPD was assessing by considering antibiotic drugs that were widely used on bacterial isolates. Almost all bacterial isolated were multidrug resistant (MDR) in both IPD and OPD, except for cefotaxime that retained potency on IPD isolates (Fig 2). The resistance patterns of bacteria towards each antibiotic on IPD vs OPD was not statistically significant with a Chi-square test analysis. Most MDR strains were seen from the OPD and almost all gram-negative bacteria showed resistance to cefotaxime, an indication for the presence of ESBL-(Fig 2) and carbapenamase-(Fig 1B) producing bacteria [9].

The association of patient gender, age group, location, and year of isolation with resistant pattern of bacteria isolates

We conducted a chi-square test to determine the link between resistance pattern of bacteria and independent variables. We found that the potency of both nitrofurantoin and cefuroxime were affected by gender. Specifically, the effectiveness of nitrofurantoin on *E. agglomerans* (p = 0.041), co-trimoxazole on coagulase negative *Staphylococci* (CoNS, p = 0.044), cefuroxime on *E. coli* (p = 0.011) and cefotaxime on *K. oxytoca* (p = 0.026) as presented in Table 1. The patient location impacted negatively on the performance of chloramphenicol and ciprofloxacin against *E. agglomerans* (p = 0.002) and *K. pneumoniae* (p = 0.021), respectively (Table 1). Further analysis revealed that the *E. aerogenes* resistance to co-trimoxazole (p = 0.005) varied



	AMP		CFX		C		CIP		SXT		GEN		NA		NIT		TET		
Bacteria	IPD	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD	OPD	KEY
C. freundii	17	83	0	75	0	100	50	50	27	73	25	75	25	75	50	50	100	0	100
C.diversus	75	25	0	0	67	33			100	0			100	0					75
CoNS	0	0	0	50	0	100	0	100	50	50	0	100			0	100	67	33	50
E. aerogenes	15	85	0	86	33	67			42	58	17	83	18	82	38	63	100	0	25
E. agglomerans	33	67	0	67	78	22	60	40	29	71	43	57	31	69	33	67	28	72	0
E. cloacae	40	60	0	67	40	60	0	100	38	62	0	100	29	71	14	86			
E. coli	35	65	0	70	14	86	33	67	41	59	44	56	30	70	36	64	22	78	
Hafnia alveii	67	33			100	0			67	33	100	0	100	0					
K. oxytoca	44	56	0	44	38	63	100	0	35	65	14	86	36	64	0	100	67	33	
K. pneumoniae	29	71	0	100	20	80	75	25	22	78	44	56	18	82	13	88	33	67	
P. mirabilis	40	60	0	50	50	50	0	100	43	57	25	75	33	67	100	0	40	60	
P. vulgaris	100	0			67	33			100	0	100	0	100	0	0	100			
P. aeruginosa	50	50			0	100	100	0	33	67	50	50	33	67					
S. marcescens	30	70	0	50	50	50	100	0	13	88	75	25	14	86	0	100	50	50	
S. aureus	50	50	0	75	38	62	9	91	32	68	0	100			14	86	28	72	
S. epidermidis							20	80	25	75							0	100	
S. saprophyticus									50	50					0	100	100	0	
Y. enterocolytica	0	100	0	100	0	100			0	100	0	100	0	100					

Fig 2. The resistance patterns in percentages of bacteria on wisely used antibiotics. The colour coded graph showed resistance pattern ranging from the least (0%, in green) to most resistant strain (100%, in red). Bacteria that were not tested on some antibiotics are shown with blank spaces. AMP: ampicillin, CFX: cefotaxime, C: chloramphenicol, CIP: ciprofloxacin, SXT: cotrimoxazole, GEN: gentamicin, NA: nalidixic acid, NIT: nitrofurantoin, TET: tetracycline, IPD: in-patient department, OPD: outpatient department, CoNS: Coagulase negative Staphylococci. Graph key shows increased colour intensity from green (0%) to red (100%).

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Table 1. The resistant pattern of some bacteria isolates with respect to patient location, year of isolation, patient gender, and specimen type.

Gender					
Microorganism	**Female**	**Male**		**p-value**	**Drug**
E. agglomerans	30.3% (10/33)	66.7% (8/12)		0.041	Nitrofurantoin
CoNS	50% (3/6)	100% (9/9)		0.044	Co-trimoxazole
E. coli	9.1% (1/11)	100% (3/3)		0.011	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
Specimen					
Microorganism	**Blood**	**Urine**		**p-value**	**Drug**
S. aureus	100% (3/3)	33.3% (15/45)		0.047	Tetracycline
Year					
Microorganism	**2019**	**2020**	**2021**	**p-value**	**Drug**
E. aerogenes	100% (10/10)	40% (2/5)	0% (0/1)	0.005	Co-trimoxazole
S. aureus		66.7% (10/15)	24.2% (8/33)	0.009	Tetracycline
S. aureus	37.9% (25/66)	18.2% (4/22)	0% (0/14)	0.002	Chloramphenicol
S. aureus	83.9% (52/62)	80.8% (21/26)	63.0% (17/27)	0.041	Co-trimoxazole

CoNS: Coagulase negative *Staphylococci*

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with year of isolation, and so was *S. aureus* resistance to tetracycline (p = 0.009), chloramphenicol (p = 0.002) and co-trimoxazole (p = 0.041). Lastly, *S. aureus* resistance to tetracycline was affected by specimen type (p = 0.047; Table 1).

Characterization of bacterial isolates from paediatric specimens

Out of the total of 500 specimens that met the inclusion criteria for this study, 102 (20.4%) were paediatric specimens. Of the 102, 46(45.1%) specimens were received from female and 56 (54.9%) from males, ranging age between 0 and 16 years with the mean average of 4.12±5.0 years. Many specimens came from in-patient department (76, 74.5%) while 26 (25.5%) came from OPD, and 37 (36.3%) specimens were analysed in the year 2019, followed by the year 2020 with 29 (28.4%) while 36 (35.3%) were processed in the year 2021 (Fig 3A). The most isolated bacterium was *S. aureus* (44, 43.1%) followed by *E. coli* (17, 16.7%), *E. agglomerans* (16, 15.7%), *K. pneumoniae* (7, 6.9%), and *K. oxytoca* (4, 3.9%; Fig 3B). Antibiotic susceptibility testing was conducted with a panel of antibiotics that are commonly used at LCH. The resistance pattern revealed that ampicillin (95.5%) was the least effective drug followed by oxacillin (87.5%), penicillin (84.8%), co-trimoxazole (82.5%), erythromycin (74.2%), and nalidixic acid (60%) whereas the most effective antibiotics were imipenem (7.7%), nitrofurantoin (29.6%) and cefotaxime (34.1%) as shown in Fig 3C. However, patient gender, patient location, specimen type and year of specimen processing had no statistical effect on the resistance pattern of bacterial isolates.

Characterization of bacterial isolates from in-patient department

Due to the over-use of various disinfectants and drugs in hospitals, bacteria species tend to be highly resistant. So, we wanted to understand the behaviour of bacterial isolates to commonly utilized antibiotics from in-patient department. Out of the 500 specimens that met the

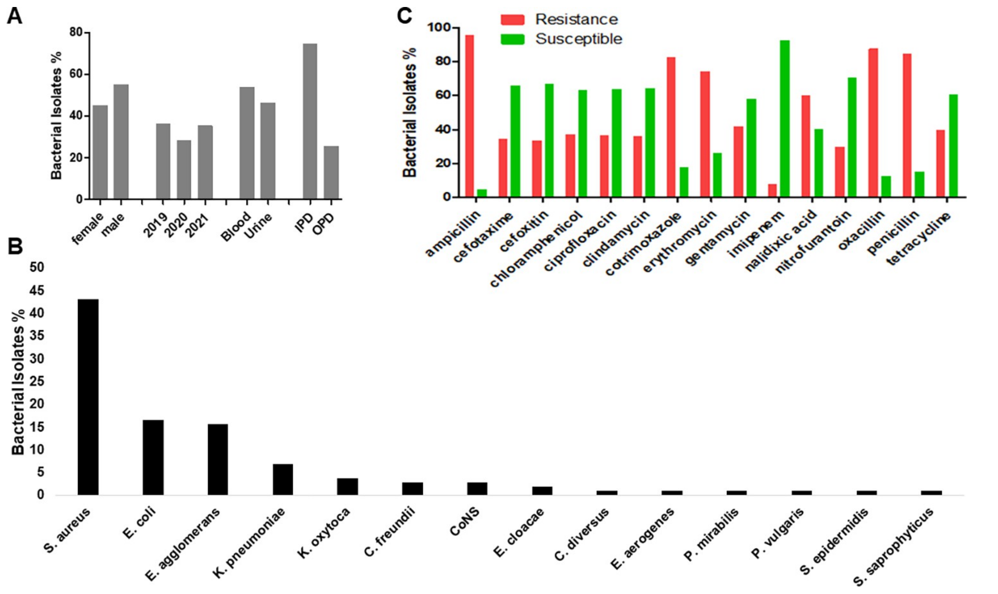

Fig 3. Characterization of bacterial isolates from paediatric specimens. (A) bacteria isolate in percentages based on patient gender, location, year of isolates, and specimen type. Blood- and uro-pathogens were the common isolates, and from IPD. (B) The common bacterial isolate (in percentages) showing the *S. aureus* as the most isolate and *S. saprophyticus* as the least isolate. (C) The susceptibility patterns (in percentages) of bacterial pathogens with imipenem, cefotaxime and nitrofurantoin retaining their potencies. IPD: in-patient department, OPD: out-patient department, CoNS: Coagulase negative *Staphylococci*.

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inclusion criteria for this study, 177 (34.5%) were specimens from in-patient departments. Of the 177, 92 (52%) specimens were received from female and 85 (48%) from males, ranging age between 0 and 80 years with the mean average of 25.5±24 years. Categorizing the age groups in 0–16 years, 17–39 years, and 40–80 years, 76 (42.9%)-, 58 (32.8%)-, and 43 (24.3%)- specimens

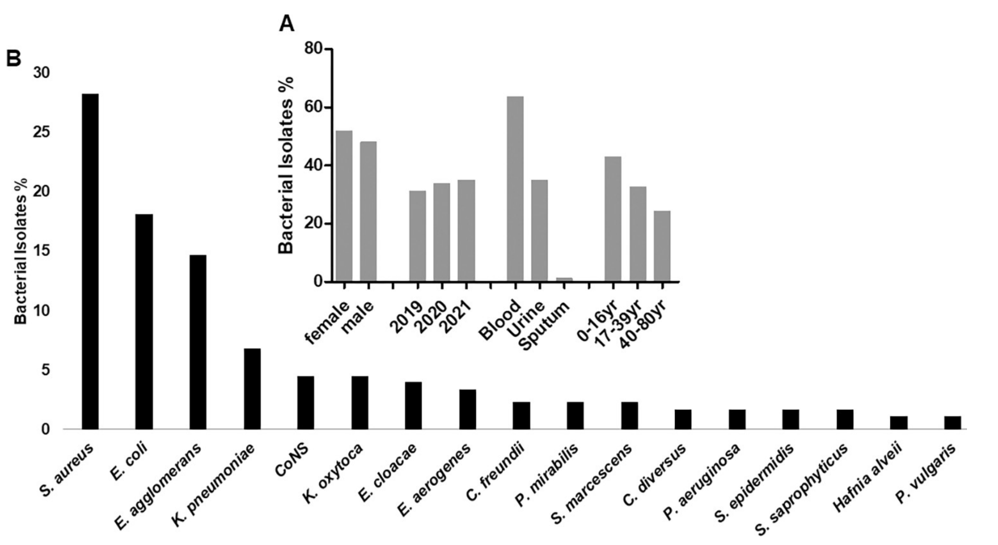

Fig 4. Characterization of bacterial isolates from in-patient department specimens. (A) Bacteria isolates in percentages based on patient gender, year of isolates, specimen type, and age category. More blood and uro-pathogens were isolated from young patients. (B) Bacteria isolate in percentages showing *S. aureus* and *P. vulgaris* as the most and least isolates, respectively. yr: year.

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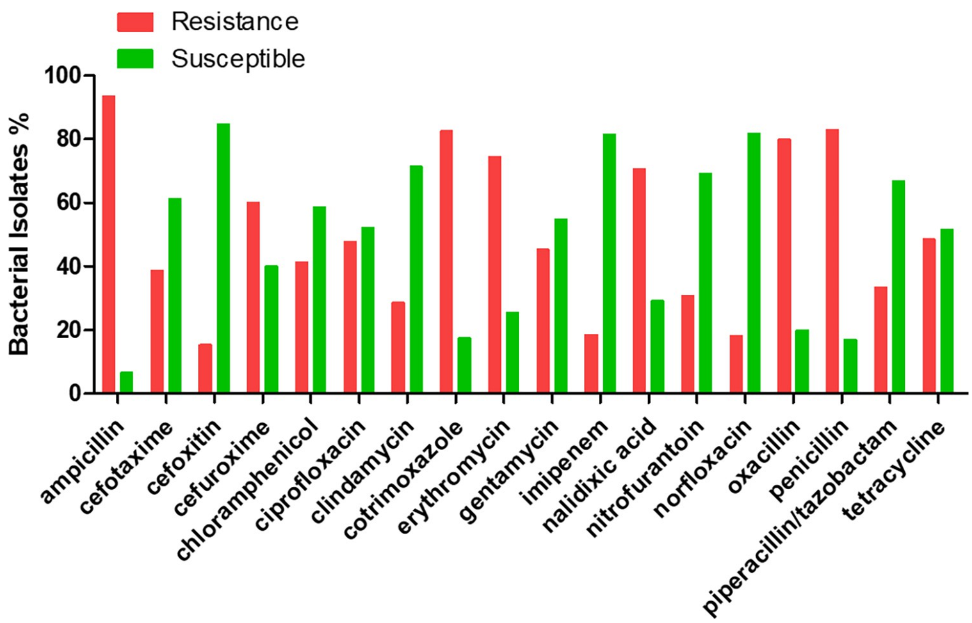

Fig 5. The susceptibility patterns of isolates from in-patient departments. Data indicates strains resistance to ampicillin, cotrimoxazole, nalidixic acid, erythromycin, oxacillin, and penicillin. While imipenem, norfloxacin, clindamycin and nitrofurantoin retained their effectiveness.

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were received, respectively (Fig 4A). For the type of specimens requested, blood specimens were the highest with 113 (63.8%) followed by urine specimen (62, 35%) and sputum (2, 1.2%; Fig 4A). Also, 62 (35%) specimens were analysed in the year 2021, followed 60 (33.69%) specimens in the year 2020, and 55 (31.1%) specimens in the year 2019 (Fig 4A). The most isolated bacterium was *S. aureus* (50, 28.2%) followed by *E. coli* (32, 18.1%), *E. agglomerans* (26, 14.7%), *K. pneumoniae* (12, 6.8%), and CoNS (8, 4.5%) as presented on Fig 4B.

Antibiotic susceptibility testing was conducted with a panel of antibiotics that are commonly used at LCH. The resistance pattern revealed that ampicillin (93.4%) was the least effective drug followed by penicillin (83%), co-trimoxazole (82.5%), oxacillin (80%), erythromycin (74.4%), nalidixic acid (70.7%), cefuroxime (60%), and tetracycline (48.5%) whereas the most effective antibiotics were norfloxacin (18.2%), imipenem (18.5%) and clindamycin (28.6%) as shown in Fig 5. The prevalence of MRSA among *S. aureus* that were tested against cefoxitin was 15.4% (2/13) whereas 84.6% (11/13) were methicillin susceptible *Staphylococcus aureus* (MSSA, Fig 5).

However, *S. aureus* resistance to chloramphenicol had a significant reduction from 2019 to 2021 (p = 0.006) whereas its resistance to penicillin was the highest in 0-16years age group (p = 0.012, Table 2).

Table 2. The resistant pattern of some IPD bacteria isolates with respect to year of isolation and patient age group.

		Year			
Microorganism	**2019**	**2020**	**2021**	**p-value**	**Drug**
S. aureus	52.9% (9/17)	22.2% (2/9)	0% (0/11)	0.006	Chloramphenicol
		Age group			
Microorganism	**0-16years**	**17-39years**	**40-80years**	**p-value**	**Drug**
S. aureus	85.5% (17/19)	100% (10/10)	42.9% (3/7)	0.012	Penicillin

https://doi.org/10.1371/journal.pgph.0000623.t002

Discussion

General characterization of bacterial isolates from routine specimens

Antimicrobial resistance (AMR) is a global public health problem that requires concerted effort from all sectors of life to control it. Due to lack of data on prevalence rates because of limited or absent antimicrobial surveillance systems and poor resources for antimicrobial susceptibility testing, AMR is a significant problem in sub-Saharan Africa [12]. Literature indicates that multidrug resistance is prevalent among gram-negative and gram-positive bacterial pathogens causing common infections irrespective of whether community- or hospital-acquired [12, 13]. The One Health concept that focusses on consequences, responses, and actions at the animal-human-ecosystems interfaces, is needed to mitigate this issue and hence deserves full support from policymakers and stakeholders. Therefore, the current study aimed to evaluate the antibiotic resistant patterns of pathogens isolated from routine laboratory specimens from 2019 to 2021 at LCH as a means of understanding the AMR trends at the hospital.

Generally, our study revealed that blood specimens were the most frequently processed specimen with 66.2% followed by urine (33%) and sputum (0.8%) indicating bacteraemia and urinary tract infections as the commonly managed infections at LCH. A similar study conducted in Ndola, Zambia found urine and blood specimens as the common processed specimens at Ndola Teaching Hospital [14]. Furthermore, many specimens came from out-patient department (64.7%), and we further noted that the influx of specimens to the laboratory reduced from 35% (for 2019 and 2020) to 30% (for 2021), conceivably because of the COVID19 pandemic that caused Government to put the country on periodic lockdown, thereby reducing the number of patients that accessed health services.

Staphylococcus aureus (28.4%) was the commonest isolate followed by *E. coli* (18.2%), *E. agglomerans* (15.2%), *K. pneumoniae* (8.6%), and the least isolated bacteria were *Hafnia alvei*, *P. vulgaris*, and *Y. enterocolytica* (0.6% apiece). This finding confirmed previous studies that had shown the rising cases of *S. aureus* infections in humans and animals in Zambia [14–17]. In a quest to understand the susceptibility patterns, the isolated organisms showed high levels of resistance to commonly utilised antibiotics. For instance, Ampicillin (93%) was the least effective drug followed by oxacillin (88%), penicillin (85.6%), co-trimoxazole (81.5%), erythromycin (71.9%), nalidixic acid (68%), ceftazidime (60%), tetracycline (55.1%), and ciprofloxacin (45.9%) whereas the most effective antibiotics were imipenem (14.5%), piperacillin/tazobactam (16.7%) and clindamycin (34.5%). The observed resistance pattern is alarming because most isolates could survive in different classes of antibiotics like penicillins and cephalosporins, macrolides, quinolones, fluoroquinolones, tetracyclines, and sulfonamides, leaving few therapeutic options like carbapenem and lincomycin. Similarly, imipenem was reported to be the most effective drug on uropathogens in India [18], and on *S. aureus* and *S. pseudintermedius* isolates from the veterinary hospitals' in- and outpatients and the environment in Zambia [15].

Several studies reveal that sepsis due to bacterial infections emanates from urinary tract infections which are more prevalent in women [19–21]. So, we expected to observe the similar trend in this study. Surprisingly, the potency of nitrofurantoin on *E. agglomerans* ($p = 0.041$), cotrimoxazole on CoNS ($p = 0.044$), cefotaxime on *K. oxytoca* ($p = 0.026$), and cefuroxime on *E. coli* ($p = 0.011$) were significantly reduced in male patients. This observation required further characterization of blood and urine isolates to clearly understand this phenomenon. Furthermore, the potency of chloramphenicol on *E. agglomerans* ($p = 0.002$) and ciprofloxacin on *K. pneumoniae* ($p = 0.021$) were significantly high among in-patients, which agreed with another study done in Kuwait [22]. Finally, cotrimoxazole potency on *S. aureus* isolates from blood specimens were significantly ($p = 0.005$) low. Therefore, the use of antibiotics especially from male admitted patients should always be proven by susceptibility testing. The resistant trend observed in this study clearly indicate possible prevalence of extended spectrum beta lactamases (ESBLs) [23], and *E. agglomerans*, *K. pneumonia* and *S. aureus* are clinically important pathogens that can develop multidrug resistance and cause difficult to treat infections in all age groups [24, 25]. Imipenem is a carbapenem that shows broad-spectrum activity against gram-positive, gram-negative, and anaerobic bacteria, and is active against cephalosporin-resistant Enterobacteriaceae producing ESBLs, whereas piperacillin/tazobactam is the combination of a fourth generation, extended-spectrum penicillin and a beta-lactamase inhibitor that is also effective against β-lactamase producing penicillin-resistant bacterial species [26, 27]. Due to the wide spread of antibiotic resistance resulting from ESBL producing *Enterobacteriaceae*, carbapenems should normally be reserved as alternative treatment for such infections [28] but our study found that both imipenem and piperacillin/tazobactam were broadly used in almost all infections. Despite imipenem being observed as the most effective antibiotics at this LCH, there could be chances of irrational use of the antibiotics subsequently posing a risk of developing carbapenem-resistant bacterial strains. Also, the resistant pattern of isolated strains suggests prevalence of ESBL-producing Enterobacteriaceae that warrant further investigations. To circumvent the issues of carbapenem resistant Enterobacteriaceae and ESBL-producing Enterobacteriaceae, antimicrobial stewardship program should be implemented to curb purported irrational use of antibiotics and enhance adherence to proposed guidelines on antimicrobial use [29]. Additionally, the need for more studies (especially molecular studies) to further assess the prevalence of carbapenemases and redesign the empiric therapy to safeguard few potent carbapenems cannot be over emphasized.

Characterization of bacterial pathogens from paediatric department

Antimicrobial resistance impacts all population and the increasing trend of drug resistant infections in infants and children is usually unrecognized while some studies have shown prevalence of ESBL producing organisms [30, 31]. Because of this scarcity of AMR data in children, we decided the characterize bacteria isolates from paediatric department specimens.

We discovered that most specimens were blood and urine that came from in-patient department (74.5%) and there were little variations in the influx of specimens as 36.3% (2019), 28.4% (2020) and 35.3% (2021) were recorded. The most isolated bacterium was *S. aureus* (43.1%) followed by *E. coli* (16.7%), *E. agglomerans* (15.7%), *K. pneumoniae* (6.9%), *K. oxytoca* (3.9%), and *C. freundii* (2.9%). The study revealed that ampicillin (95.5%) was the least effective drug followed by oxacillin (87.5%), penicillin (84.8%), co-trimoxazole (82.5%), erythromycin (74.2%), and nalidixic acid (60%) whereas the most effective antibiotics were imipenem (7.7%), nitrofurantoin (29.6%) and cefotaxime (34.1%). An increasing prevalence of uropathogen resistance to ceftriaxone, cefuroxime, amoxicillin/clavulanate, and ampicillin had been reported in children [32]. Therefore, prescribing amoxiclav, cotrimoxazole and nalidixic acid in children should be done cautiously with susceptibility testing.

Characterization of bacterial isolates from in-patient department

Hospital acquired infections (HAIs) are of major safety concern for both health care providers and the patients [33]. These infections are usually acquired after hospitalization and manifest 48–72 hours after admission to the hospital. Even though it was difficult to determine whether the infections were hospital-acquired or not, due to the retrospective nature of our study, we thought of having a glimpse on the resistant levels of bacteria isolated from IPD. We noticed that more blood and urine specimens came from female patients. The most isolated bacterium was *S. aureus* (28.2%), followed by *E. coli* (18.1%), *E. agglomerans* (14.7%), *K. pneumoniae*

(6.8%), CoNS (4.5%), and *K. oxytoca* (4.5%). The study revealed that ampicillin (93.4%) was the least effective drug followed by penicillin (83%), co-trimoxazole (82.5%), oxacillin (80%), erythromycin (74.4%), nalidixic acid (70.7%), cefuroxime (60%), and tetracycline (48.5%) whereas the most effective antibiotics were norfloxacin (18.2%), imipenem (18.5%) and clindamycin (28.6%). The picture of multidrug resistant strains was observed amongst IPD specimens and this calls for an in-depth analysis of antibiotic susceptibility patterns of nosocomial pathogens. The prevalence of gram-negative HAI- and carbapenem resistant- pathogens have been reported across the globe [34, 35] and the cases are steadily increasing, posing a risk on the cost and effective management of HAIs. Therefore, antimicrobial stewardship programs [29, 33] should be implemented in referral hospitals in Zambia and beyond.

In conclusion, our study has revealed the proportion of antibiotic resistance of commonly isolated bacteria at LCH and the importance of periodic monitoring of antibiotic resistance patterns in hospitals. Antibiogram studies can aid in selecting an effective antibiotic therapy, tracking local antibiotic resistance trends, and identifying antimicrobial stewardship initiatives and targets for education and improvement. The study discovered the presence of MDR strains among adult and paediatric in-patients and out-patients, an indication that the choice of antibiotic therapy at LCH may be reducing. Therefore, more studies to establish the prevalence of ESBL- and carbapenemase- producing bacteria for empiric therapy redesigning are warranted as monitoring data on antimicrobial susceptibility of common bacterial organisms is crucial for decision-making and quick detection of AMR at hospital levels. Moreover, identifying specific hospital wards and clinics with huge antibiotic resistance burden will be helpful in strategizing on an effective infection prevention method, as it was not established in our study.

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Author Contributions

Conceptualization: Kingsley Kamvuma, Warren Chanda.

Formal analysis: Kingsley Kamvuma, John Amos Mulemena, Christopher Newton Phiri, Warren Chanda.

Investigation: Thresa N. Mwansa, Kingsley Kamvuma, John Amos Mulemena.

Methodology: Thresa N. Mwansa, Kingsley Kamvuma, Warren Chanda.

Supervision: Warren Chanda.

Validation: Christopher Newton Phiri.

Writing – original draft: Thresa N. Mwansa, Warren Chanda.

Writing – review & editing: Kingsley Kamvuma, John Amos Mulemena, Christopher Newton Phiri, Warren Chanda.

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