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Original Article **Open Access**

Antibiotic resistance profiles of uropathogenic bacterial isolates in Haut-Sassandra Region, Côte d'Ivoire from January 2019 to December 2022

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Abstract:

Background: The escalating issue of bacterial resistance is a profound universal peril. This looming crisis has evolved from a mere forecast to a tangible reality globally. Urinary tract infections (UTIs) significantly influence antibiotic prescriptions in primary care, thus crucially impacting the selective pressure and the emergence of antibiotic-resistant bacteria. A profound comprehension of the microorganisms involved in UTIs and their resistance patterns is crucial, particularly in Daloa city, Côte d'Ivoire. This research aims to review the antibiotic resistance profiles of uropathogens isolated from patients in the Regional Hospital Center (CHR) of Daloa, Côte d'Ivoire from January 2019 to December 2022.
Methodology: This was a descriptive cross-sectional study of 1,513 patients whose voided urine samples were received at the Bacteriology-Virology Laboratory of CHR for cyto-bacteriological examination and aerobic culture using standard microbiological protocols over a period of 4 years. Bacterial isolates were routinely identified by colony morphology, Gram staining reaction and conventional biochemical tests. The antibiotic susceptibility of the bacterial isolates was determined by the agar diffusion method and interpreted following the Antibiogram Committee of the French Society of Microbiology (CASFM) guidelines.
Results: Of the 1,513 patient urine samples examined, 246 (16.3%) were positive for microbial organisms, 216 (14.3%) were positive for significant bacterial isolates, 9 (0.6%) were positive for fungi, and 21 (1.4%) were positive for ova of *Schistosoma haematobium*. Among the samples with significant bacteriuria, 91.2% were due to Gram-negative bacilli, 5.9% to Gram-positive cocci, and 2.9% to Gram-negative cocci. *Escherichia coli* was the most predominant bacterial pathogen, accounting for 73.2% of the isolates. Antibiotic susceptibility testing showed high *in vitro* resistance of the bacterial isolates to tested antibiotics, with Enterobacteriaceae exhibiting resistance rate between 56.0% for nalidixic acid (NAL) and 67.0% for amoxicillin/clavulanic acid (AMC). *Pseudomonas aeruginosa* isolates exhibited 50.0% resistance rate to ceftazidime (CAZ), ciprofloxacin (CIP), and ticarcillin (TIC) while *Staphylococcus* isolates demonstrated 100.0% resistance rate to ofloxacin (OFX), clindamycin (CMN), erythromycin, trimethoprim/sulfamethoxazole (SXT), and fusidic acid (FA). The extended-spectrum beta-lactamase (ESBL)-producing isolates were identified in 15.1% of the Enterobacteriaceae.
Conclusion: The high prevalence of antibiotic resistant bacterial isolates from significant bacteriuria in our study highlights the pressing need for the formulation and implementation of strategies to address this potential public health menace. The findings of our study may be useful for healthcare authorities to plan strategic interventions that will assist in optimizing the management of bacteriuria and UTI in the city of Daloa.

Keywords: Urinary tract infection, bacterial resistance, antibiotic, ESBL, Enterobacteriaceae

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Profils de résistance aux antibiotiques des isolats bactériens uropathogènes dans la région du Haut-Sassandra, Côte d'Ivoire de janvier 2019 à décembre 2022

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Résumé:

Contexte: Le problème croissant de la résistance bactérienne constitue un grave péril universel. Cette crise imminente est passée d'une simple prévision à une réalité tangible à l'échelle mondiale. Les infections des voies urinaires (IVU) influencent considérablement les prescriptions d'antibiotiques en soins primaires, ayant ainsi un impact crucial sur la pression sélective et l'émergence de bactéries résistantes aux antibiotiques. Une compréhension approfondie des micro-organismes impliqués dans les infections urinaires et de leurs modèles de résistance est cruciale, en particulier dans la ville de Daloa, en Côte d'Ivoire. Cette recherche vise à examiner les profils de résistance aux antibiotiques des uropathogènes isolés chez les patients du Centre Hospitalier Régional (CHR) de Daloa, Côte d'Ivoire de janvier 2019 à décembre 2022.
Méthodologie: Il s'agit d'une étude transversale descriptive portant sur 1513 patients dont les échantillons d'urine vidés ont été reçus au Laboratoire de Bactériologie-Virologie du CHR pour examen cyto-bactériologique et culture aérobie selon des protocoles microbiologiques standards sur une période de 4 ans. Les isolats bactériens ont été systématiquement identifiés par la morphologie des colonies, la réaction de coloration de Gram et les tests biochimiques conventionnels. La sensibilité aux antibiotiques des isolats bactériens a été déterminée par la méthode de diffusion sur gélose et interprétée selon les directives du Comité Antibiogramme de la Société Française de Microbiologie (CASFM).
Résultats: Sur les 1513 échantillons d'urine de patients examinés, 246 (16,3%) étaient positifs pour les organismes microbiens, 216 (14,3%) étaient positifs pour des isolats bactériens significatifs, 9 (0,6%) étaient positifs pour des champignons et 21 (1,4%) étaient positifs pour ovules de *Schistosoma haematobium*. Parmi les échantillons présentant une bactériurie significative, 91,2% étaient dus à des bacilles à Gram négatif, 5,9% à des coques à Gram positif et 2,9% à des coques à Gram négatif. *Escherichia coli* était le pathogène bactérien le plus prédominant, représentant 73,2% des isolats. Les tests de sensibilité aux antibiotiques ont montré une résistance in vitro élevée des isolats bactériens aux antibiotiques testés, les Enterobacteriaceae présentant un taux de résistance compris entre 56,0% pour l'acide nalidixique (NAL) et 67,0% pour l'amoxicilline/acide clavulanique (AMC). Les isolats de *Pseudomonas aeruginosa* présentaient un taux de résistance de 50,0% à la ceftazidime (CAZ), à la ciprofloxacine (CIP) et à la ticarcilline (TIC), tandis que les isolats de *Staphylococcus* présentaient un taux de résistance de 100,0% à l'ofloxacine (OFX), la clindamycine (CMN), l'érythromycine, le triméthoprime/ sulfaméthoxazole (SXT) et l'acide fusidique (FA). Les isolats producteurs de bêta-lactamases à spectre étendu (BLSE) ont été identifiés chez 15,1% des Enterobacteriaceae.
Conclusion: La forte prévalence d'isolats bactériens résistants aux antibiotiques provenant d'une bactériurie importante dans notre étude souligne le besoin urgent de formuler et de mettre en œuvre des stratégies pour faire face à cette menace potentielle pour la santé publique. Les résultats de notre étude pourraient être utiles aux autorités sanitaires pour planifier des interventions stratégiques qui contribueront à optimiser la gestion de la bactériurie et des infections urinaires dans la ville de Daloa.

Mots-clés: Infection des voies urinaires, résistance bactérienne, antibiotique, BLSE, Enterobacteriaceae

Introduction:

The incidence of urinary tract infections (UTIs) caused by multidrug-resistant pathogens is escalating at an alarming rate globally (1,2), as these infections lead to extensive antibiotic prescriptions in primary care, contributing significantly to the selection pressure of bacterial resistance to antibiotics (3). The rising rates of antibiotic resistance have resulted in substantial morbidity, mortality, and increased healthcare costs (4). Economically, bacterial resistance leads to extension of hospital stays, necessitating more intensive care and costlier medications (5). Moreover, in low-income countries, the lack of knowledge about proper use, non-adherence to prescription protocols, and the abusive use of antibiotics often lead to increased phenomena of bacterial resistance to these molecules (6).

Microbial resistance to antibiotics is one of the top ten global threats to public health. According to the World Health Organization (WHO), sub-Saharan Africa is the most affected by this problem, where in 2019, 1.27 million deaths were directly attributable to bacterial resistance (7). Given this situation, it is imperative to deepen the understanding of the epidemiology and antibiotic resistance of uropathogens to ensure rational and regular surveillance of their sensitivity to antibiotics to enhance clinical outcomes. Therefore, our aim is to analyze the antibiotic resistance profiles of uropathogens from patients at the Regional Hospital Center (CHR) of Daloa, Côte d'Ivoire over a four-year period (2019 to 2022).

Materials and method:

Study setting and design:

This research was a descriptive cross-sectional study conducted in the Bacteriology-Virology Laboratory of the Regional Hospital Center (CHR) of Daloa (Côte d'Ivoire), over a period of 4 years (January 2019 to December 2022).

Urine collection and microbiological analysis:

From 2019 to 2022, a total of 1,513

urine samples were received and routinely processed at the Bacteriology-Virology Laboratory of CHR in Daloa, Côte d'Ivoire. Upon reception, the initial analysis included a macroscopic examination to identify characteristics such as appearance (clear, cloudy, purulent, haematuria, presence of sediment). This was followed by microscopic examination, which entailed a wet mount to detect cells (leukocytes, erythrocytes, epithelial cells), bacteria, ova of parasites (e. g. nematodes), yeasts, and nitrites, along with direct Gram stain.

Urine samples exhibiting significant leukocyturia were cultured using the quantitative calibrated loop method. For this, 10 µl (0.01 ml) of the sample was spread onto agar media and incubated at 37°C for 18 to 24 hrs. Nutrient agar or Uriselect medium were used for the general enumeration of urinary pathogens. The isolation of different species was facilitated by selective media such as Cetrimide agar for *Pseudomonas*, Chapman medium for *Staphylococcus*, Sabouraud agar with chloramphenicol for fungal isolation, eosin methylene blue (EMB) agar for Enterobacteriaceae, and Hektoen agar for *Salmonella*.

After culture incubation, quantitative bacterial colony counting was conducted. The interpretation of urine cultures adhered to the criteria defined by Kass in 1956, which includes a homogeneous culture (monomicrobial infection), significant leukocyturia (≥ 10^4 leukocytes/ml of urine), and significant bacteriuria (≥ 10^5 bacteria/ml of urine). Bacterial counts of 10^3 CFU/ml or 10^4 CFU/ml was also indicative of infections, particularly in samples from non-catheterized paraplegic men, women with cystitis, cases of high diuresis under antibiotic treatment or when slow-growing bacteria are present.

Following bacterial enumeration, Gram stain was performed on the colonies. Conventional biochemical such as oxidase and catalase were conducted on colonies from the ordinary agar. Enterobacteriaceae were characterized using the reduced Le Minor tray, *Staphylococcus* isolates were identified by DNase, mannitol fermentation, and motility tests, *Enterococcus* by haemolysis pattern, and *Pseudomonas* by oxidase and motility tests.

Antibiotic sensitivity testing:

From a 24-hour culture of the test isolates, a bacterial suspension was prepared in 2 ml of 0.85% NaCl solution to achieve the turbidity equivalent to 0.5 McFarland standard. The inoculum density was adjusted using a densitometer. Subsequently, Mueller Hinton (MH) agar was inoculated, followed by the placement of antibiotic-impregnated disks (as listed in Table 1). After incubation at 37°C in aerobiosis for 18 to 24 hrs, readings were taken, and results were interpreted according to the Antibiogram Committee of the French Society of Microbiology (CASFM) guidelines.

Phenotypic confirmation of extended-spectrum β-lactamase (ESBL) production:

Phenotypic confirmation of isolates producing extended-spectrum β-lactamases (ESBLs) was carried out using the double disc synergy test of amoxicillin-clavulanic acid with either of cefotaxime, ceftriaxone, or aztreonam arranged on Müeller-Hinton agar that has been pre-inoculated with the test isolates. The characteristic "champagne cork" or "funnel" zones appearing between discs of amoxicillin-clavulanic acid and the cephalosporin phenotypically confirms an isolate to be ESBL producer (8).

Data analysis:

Data were analysed using descriptive statistics on the R software (version R64 × 4.3.1), with *p* values of less than 0.05 considered statistically significant.

Results:

Prevalence of pathogens isolated in urine at CHR of Daloa:

In total, 1,513 urinary samples were received at the Bacteriology-Virology Laboratory of the CHR of Daloa from 2019 to 2022. Among these, microbial pathogens were recovered from 246 samples, giving an overall prevalence of positive urine cultures (PUCs) of 16.3%, while significant bacteria were isolated from 216 (14.3%) samples, fungi (*Candida* sp.) from 9 (0.6%) samples, and oval of nematodes (*Schistosoma haematobium*) from 21 (1.4%) samples (Fig 1).

Table 1: List of antibiotics tested on the bacterial isolates

Antibiotic class	Antibiotic name	Abbreviation	Content (µg)
Aminoglycosides	Amikacin	AKN	30
	Gentamicin	GMN	10
Beta-lactams	Cefuroxime	CXM	30
	Amoxicillin	AMX	25
	Amoxicillin/clavulanic acid	AMC	20/10
	Ticarcillin	TIC	75
	Cefepime	FEP	30
	Cefotaxime	CTX	30
	Cefoxitin	FOX	30
	Ceftriaxone	CRO	30
	Ceftazidime	CAZ	10
	Aztreonam	ATM	30
Carbapenem	Imipenem	IPM	10
Fluoroquinolones	Ciprofloxacin	CIP	5
	Nalidixic acid	NAL	30
	Ofloxacin	OFX	5
Macrolide-Lincosamide	Erythromycin	ERY	15
	Clindamycin	CMN	2
Sulphonamides	Trimethoprim/sulfamethoxazole	SXT	1.25/23.75
Others	Fosfomycin	FOS	200
	Fusidic acid	FA	10
	Rifampicin	RA	5

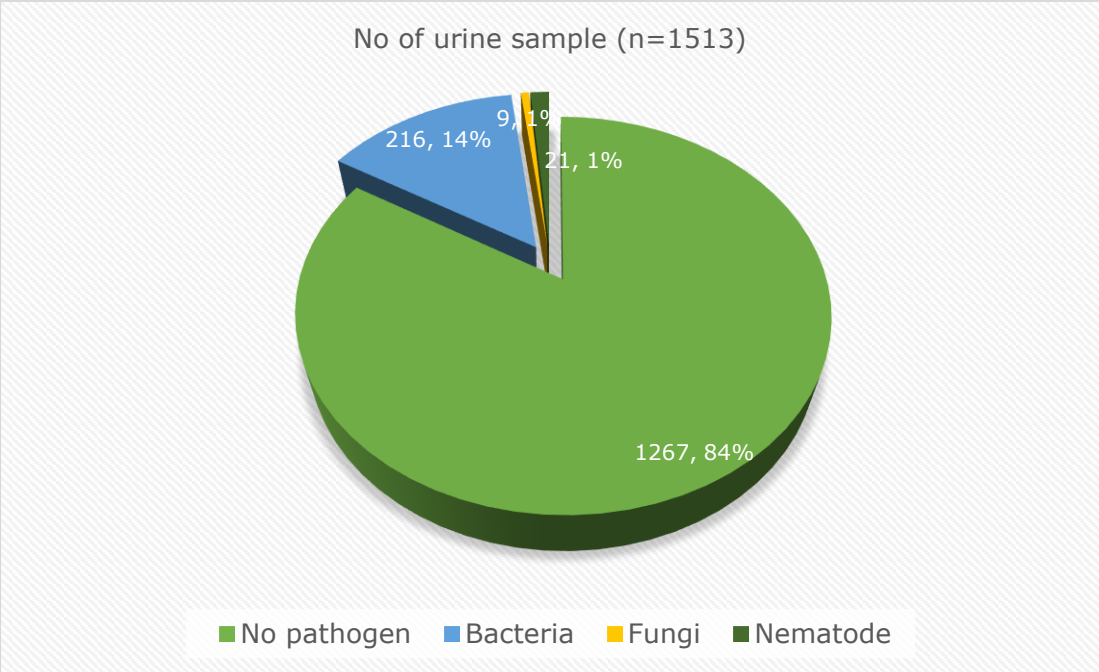

Fig 1: Prevalence of microbial pathogens from urinary tract infections in Daloa (2019 to 2022)

Distribution of bacteria species isolated from urine by Gram stain and biochemical characteristics:

Based on Gram staining reaction, significant bacteriuria was caused by Gram-negative bacilli (91.2%), Gram-positive cocci (5. 9%) and Gram-negative cocci (2.9%) (Fig 2).

Fig 3 depicts the frequency of bacterial isolates, which shows that *Escherichia coli* was the most frequently isolated with 73.2%, followed by unknown Enterobacteriaceae (4.5%), *Klebsiella* sp. (4.5%), *Klebsiella pneumoniae* (4.0%) and other bacterial isolates (13.8%).

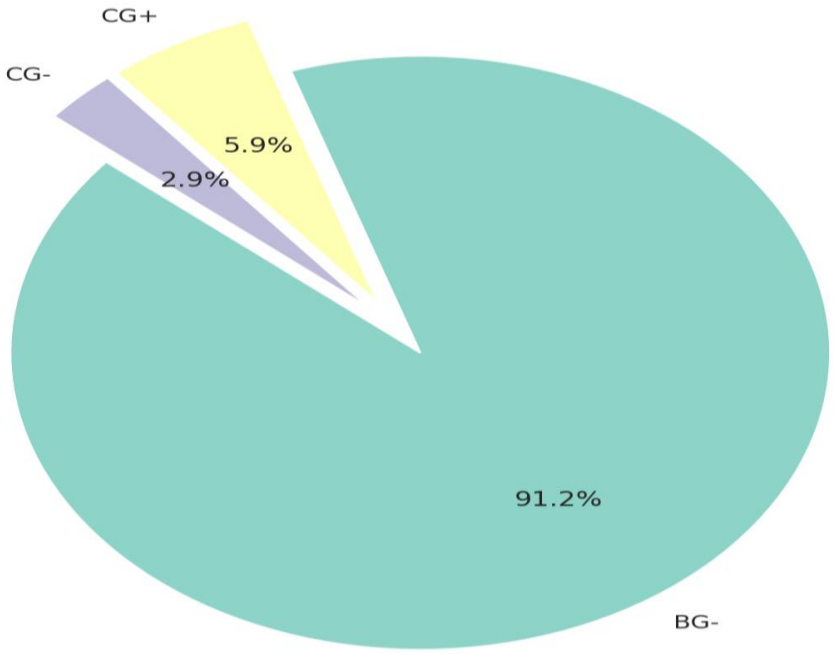

Fig 2: Frequency distribution of bacteria isolates based on Gram staining reaction

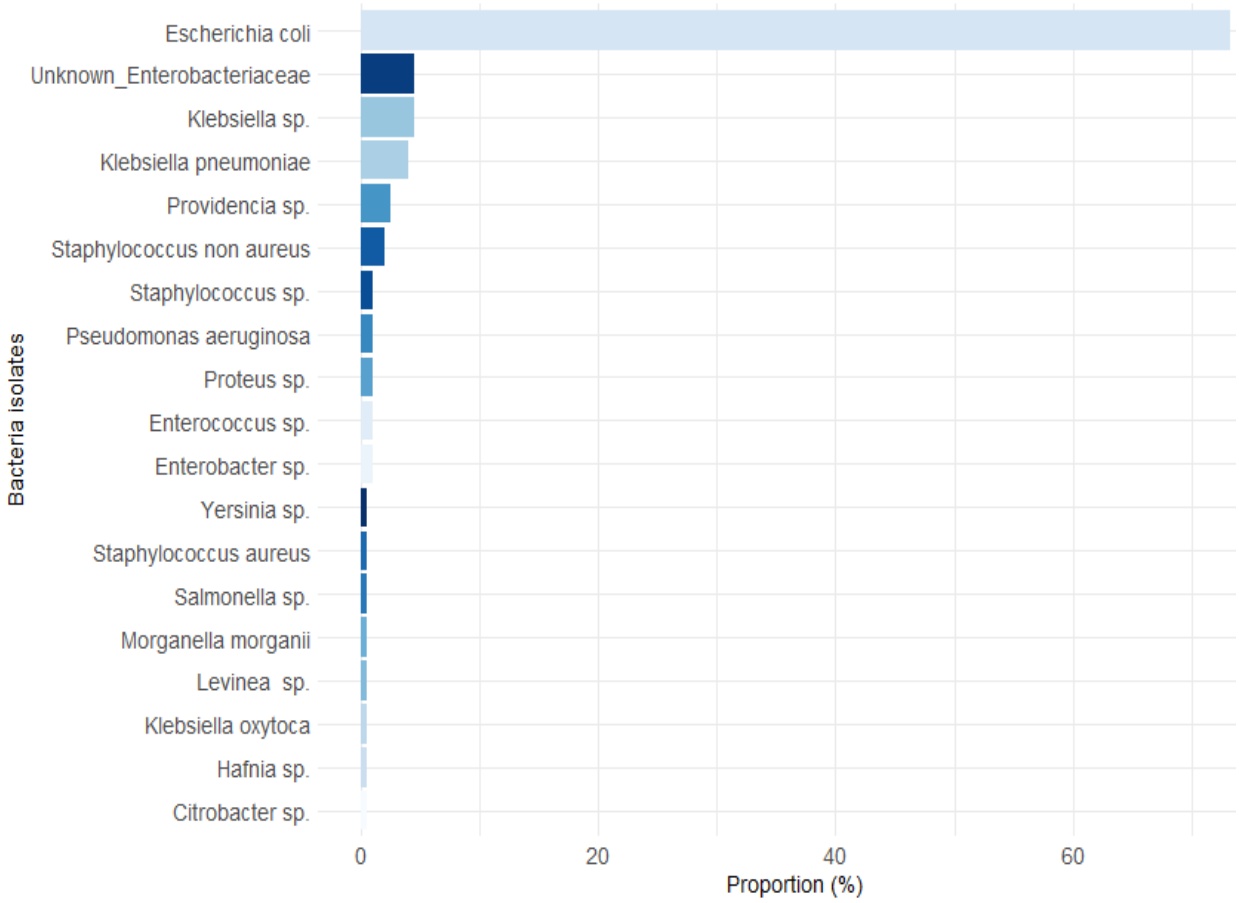

Fig 3: Frequency distribution of bacterial isolates of urinary tract infections.

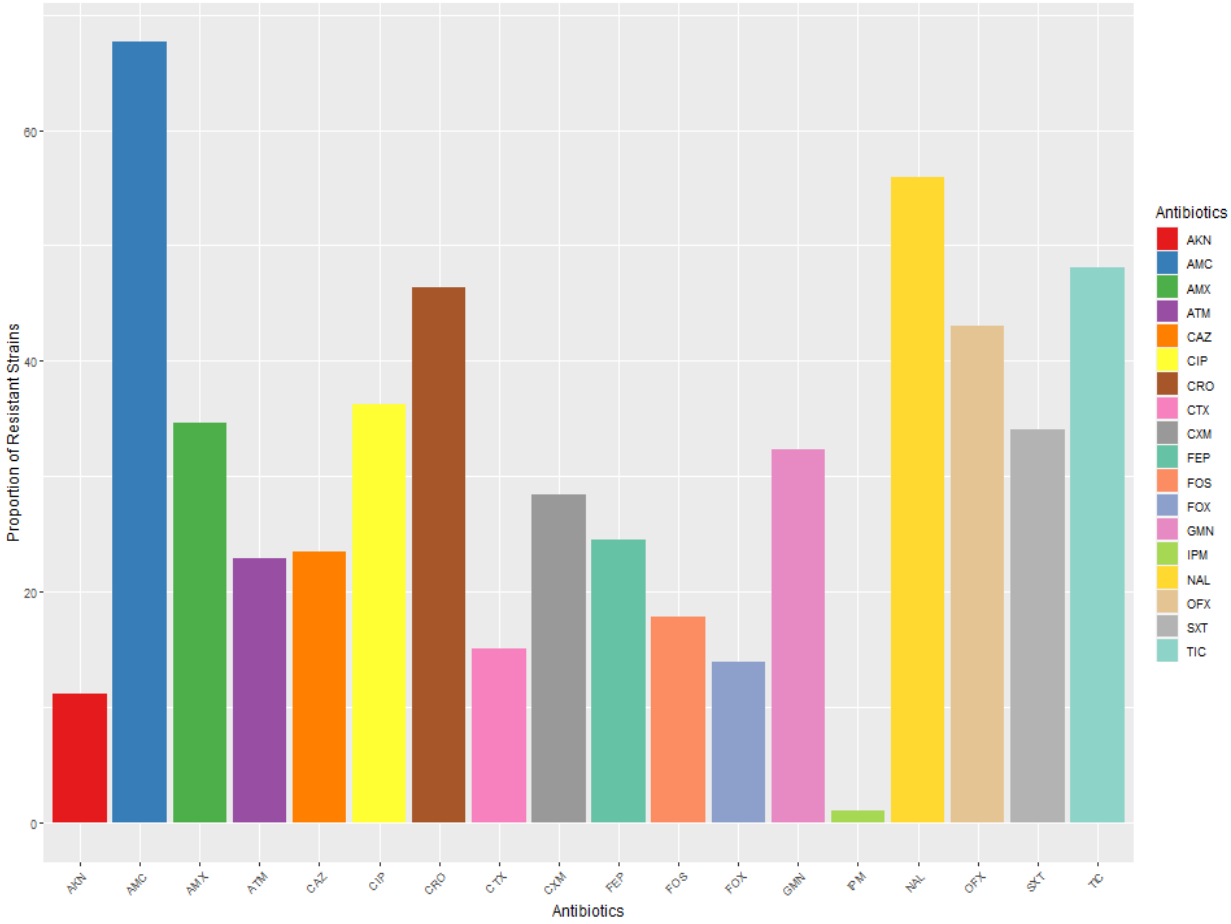

Fig 4: Resistance of Enterobacteriaceae to standard antibiotics.

Antibiotic susceptibility results of Enterobacteriaceae:

Fig 4 showed that the Enterobacteriaceae (n=179) demonstrated *in vitro* resistance to most of the antibiotics tested. However, carbapenem (represented by imipenem) showed high *in vitro* inhibitory activity (with 95% sensitivity and only 5% resistance rate) against the isolates over the 4-year period. The highest resistance rates were recorded for amoxicillin/clavulanic acid (AMC) and nalidixic acid (NAL) with resistance rates of 67.0% and 56.0% respectively.

Resistance of Enterobacteriaceae isolates of urinary tract infections to standard antibiotics:

The results of antimicrobial susceptibility showed high resistance rates to all tested antibiotics. Specifically, *Enterobacter* species were 100.0% resistant to fosfomycin (FOS), amoxicillin/clavulanic acid (AMC), trimethoprim/sulfamethoxazole (SXT), and cefoxitin (FOX). However, they were 100.0% sensitive to ciprofloxacin (CIP), cefepime (FEP), gentamicin (GMN), imipenem (IPM), and cefotaxime (CTX). For *E. coli* isolates, resistance was pronounced to ticarcillin (TIC) with a rate of 91.0%, amoxicillin (AMX) 85.0%, trimethoprim/sulfamethoxazole (SXT) 84.0%, and nalidixic acid (NAL) 82%. However, they were considerably sensitive to IPM (99.0%).

For *Klebsiella pneumoniae*, resistance rate to AMX and TIC was 100.0%, while to GMN and FOS, the rates were 75% and 67% respectively. Conversely, resistance rate to IMP, aztreonam (ATM), and CTX was 0%. The resistance rate of *Klebsiella* sp., was 100.0% to CXM, TIC, SXT, AMX, and ceftazidime (CAZ) but were 100.0% sensitive to IMP, amikacin (AKN) and cefuroxime (CTX).

For *Proteus* sp., 50.0% of the isolates were resistant to NAL, TIC and SXT. For *Providencia* sp., 100.0% were resistant to CIP, TIC, SXT and AMX. For unknown Enterobacteriaceae, 100.0% were resistant to AMX and CTX, and 80.0% to OFX. In contrast, they were 100% sensitive to IMP, GMN, and AKN.

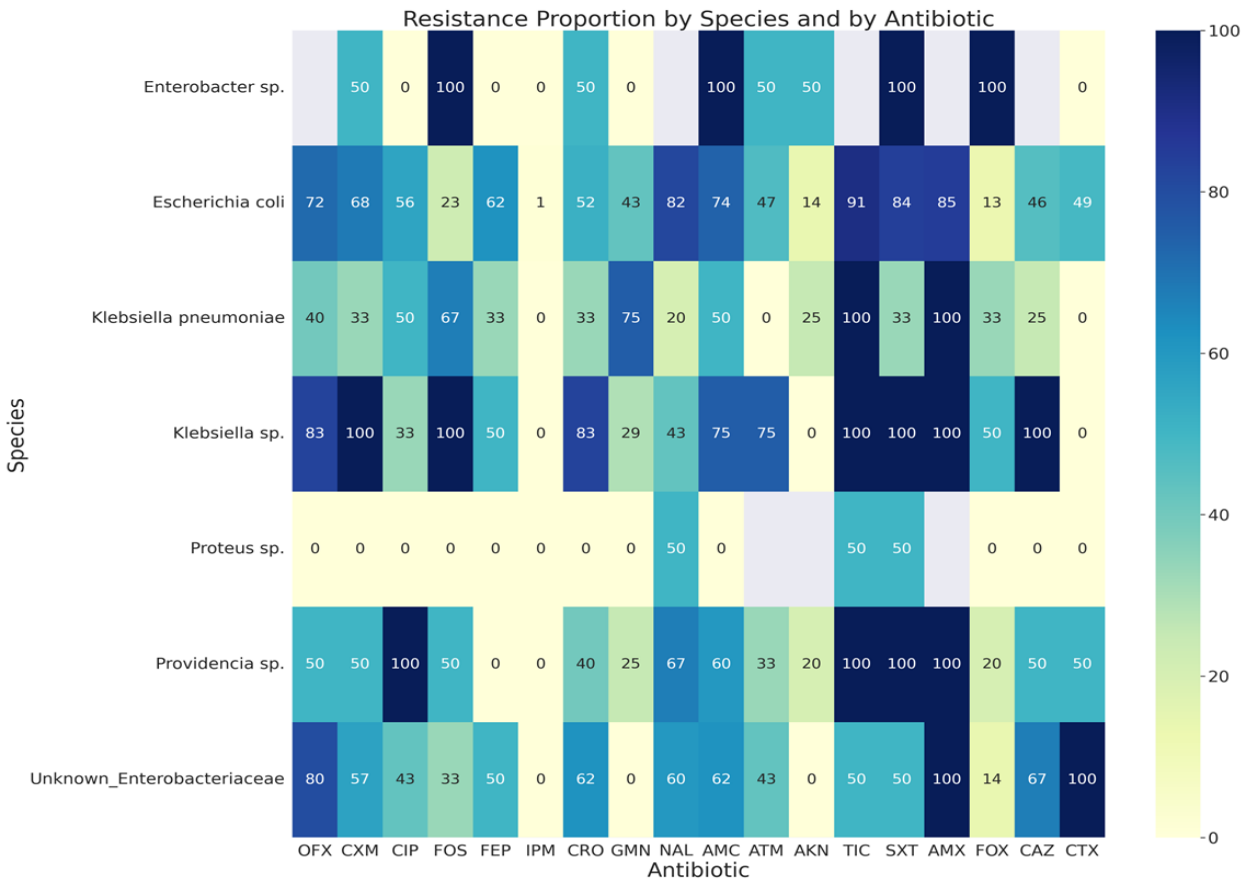

Fig 5: Resistance of Enterobacteriaceae isolates from urinary infections to common antibiotics.

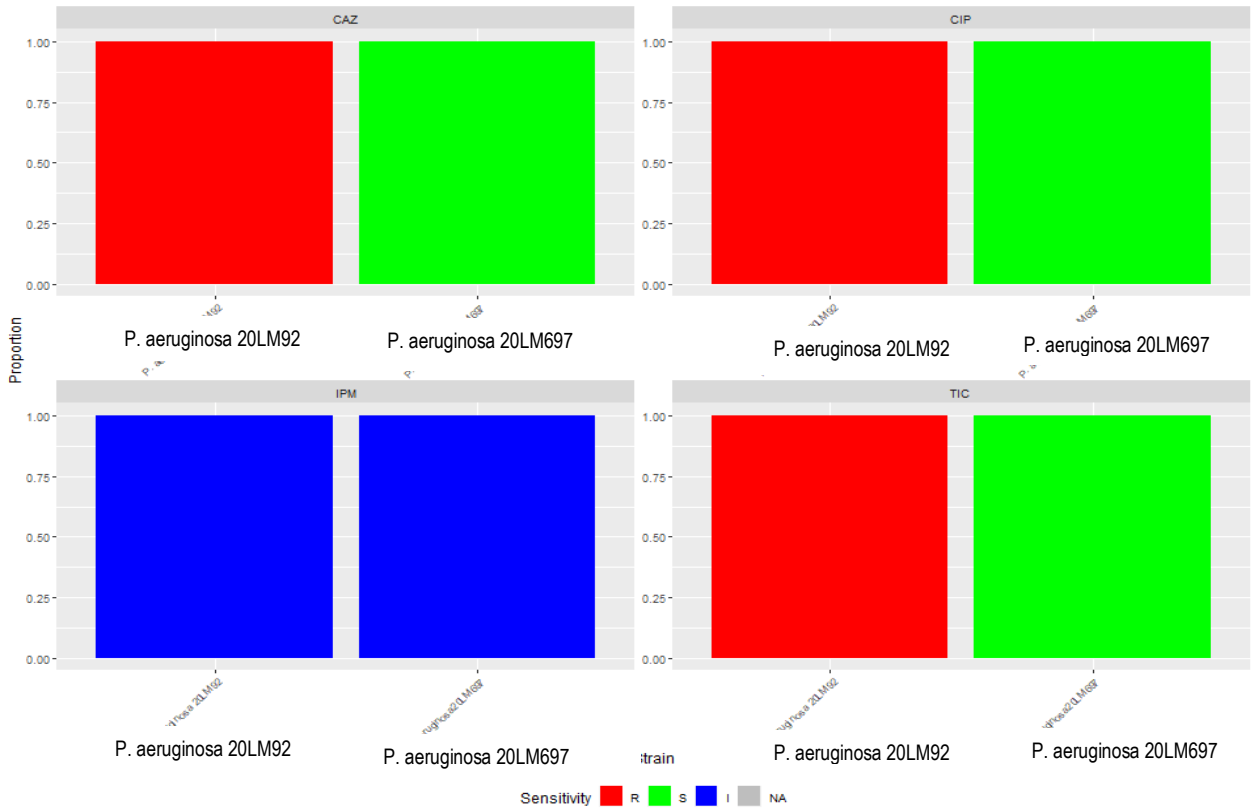

Fig 6: Resistance of *Pseudomonas aeruginosa* isolates to standard antibiotics.

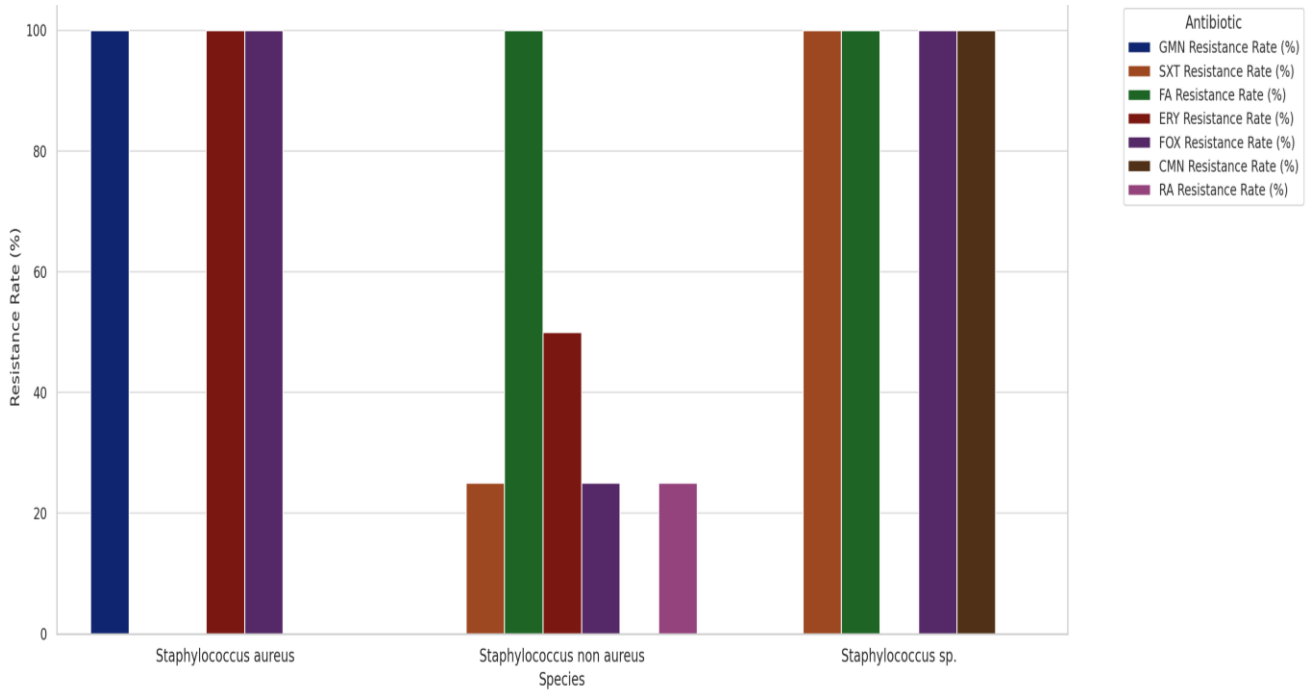

Fig 7: Resistance of *Staphylococcus* isolates to standard antibiotics.

Resistance of *Pseudomonas aeruginosa* isolates to standard antibiotics:

Fig 6 shows the resistance patterns of *Pseudomonas aeruginosa* isolates (n=2) to standard antibiotics, with 50.0% of the isolates resistant to ceftazidime (CAZ), ciprofloxacin (CIP), and ticarcillin (TIC) and 100.0% of the isolates with intermediate resistance to imipenem (IPM).

Resistance of *Staphylococcus* isolates to standard antibiotics:

The result of the antibiotic sensitivity test of the *Staphylococcus* isolates is presented in Fig 7, which showed that 100.0% of the isolates were resistant to erythromycin (ERY), clindamycin (CMN), ofloxacin (OFX), and trimethoprim/sulfamethoxazole (SXT). Also, the non-aureus *Staphylococcus* isolates were resistant to fusidic acid (FA) and ERY with 100.0% and 66.7% rates respectively. For *Staphylococcus aureus* isolates, they were 100.0% resistant to fusidic acid (FA), gentamicin (GMN), cefoxitin (FOX) and erythromycin (ERY).

Prevalence of observed resistance phenotypes resulting from antibiotic resistance in isolated bacterial isolates:

Over the 4-year period, the antibiotic sensitivity and resistance tests performed at the Bacteriology-Virology Laboratory at CHR of Daloa identified a single resistance phenotype, extended-spectrum beta-lactamase (ESBL)-producing strains among the Enterobacteriaceae isolates. This phenotype was identified in 27 of the 179 Enterobacteriaceae isolates, representing a rate of 15.1%. *Escherichia coli* accounted for 81.5% (22/27) of the ESBL-producing isolates, 14.8% (4/27) were unidentified Enterobacteriaceae, and 3.7% (1/27) were *Klebsiella* sp.

Discussion:

Urinary tract infection is a condition characterized by the inflammation of the urinary pathways resulting from abnormal colonization of detrimental microorganisms (9). Discerning the microbial aetiology and resistance patterns of these microorganisms to conventional antibiotics is pivotal to guide clinical decisions more accurately. This study, spanning four years (2019 to 2022), showed that UTIs in Daloa are predominantly bacterial in nature, with significant bacteriuria accounting for 14.3% (216/1513) of the UTI cases, followed by infections due to *Schistosoma haematobium* (1.4%,21/1513) and candiduria (0.6%,9/1513).

The predominance of bacteriuria in UTI as observed in our study has been reported by several authors (10,11). This is attributed to several factors including age, frequent sexual activity, inadequate or excessive hygiene, menopause, and homosexuality (12). The results indicated a predominant incidence of Gram-negative bacilli accounting for 91.2% of bacteriuria, with *E. coli* being the most prevalent species with 73.2%. The high prevalence of *E. coli* is corroborated by other studies (13), and could be attributed to specific factors such as presence of bacterial adhesins in *E. coli* which facilitate its binding to the urinary epithelium (14,15].

The results of antibiotic susceptibility test for the Enterobacteriaceae in particular

revealed significant resistance to all tested antibiotics, with the highest resistance to amoxicillin/clavulanic acid (67,0%), followed by nalidixic acid (56.0%), ticarcillin (48.0%), and ceftriaxone (47.0%). *Escherichia coli* showed pronounced resistance to ticarcillin (91.0%), amoxicillin (85.0%) and trimethoprim/sulfamethoxazole (84.0%). *Enterobacter* sp. isolates also exhibited profound resistance (100%) to several antibiotics including fosfomycin, amoxicillin/clavulanic acid, trimethoprim/sulfamethoxazole and cefoxitin. The non-Enterobacteriaceae isolates such as *Pseudomonas aeruginosa* also exhibited 50.0% resistance to ceftazidime, ciprofloxacin and ticarcillin, and *Staphylococcus* sp. showed 100.0% resistance rate to ofloxacin, clindamycin, erythromycin and trimethoprim/sulfamethoxazole, while the non-aureus *Staphylococcus* isolates showed 100.0% resistance to fusidic acid and 66.7% to erythromycin. These high resistance rates could be attributed to the selective pressure from extensive and often inappropriate use of broad-spectrum antibiotics in both hospital and community settings (16).

Our study primarily identified ESBL resistance phenotype among the Enterobacteriaceae, with a prevalence of 15.1%. *Escherichia coli* alone accounted for 81.5% of the ESBL-producing Enterobacteriaceae, while other unidentified Enterobacteriaceae isolates accounted for 14.8% and *Klebsiella* sp. accounted for 3.7%, findings that are in agreement with reports of other studies (17,18,19). Our study provides crucial insights into the understanding of the microbial aetiology of UTIs and their antibiotic resistance patterns to available antibiotics in Daloa, which can assist healthcare authorities in devising effective strategies for the management of significant bacteriuria and UTIs in the region.

Conclusion:

The prevalence of significant bacteriuria in our study over a 4-year period in Daloa, Cote D'Ivoire, is 14.3%, while candiduria and urinary schistosomiasis constituted 0.6% and 1.4% respectively. Bacteriuria was predominantly caused by Gram-negative bacilli (91.2%), with *E. coli* being the most prevalent isolate (73.2%). The ESBL phenotype was detected in 15.1% of the Enterobacteriaceae, with *E. coli* comprising 81.5%, *Klebsiella* sp. 3.7%, and unknown Enterobacteriaceae 14.8%. The *in vitro* resistance rates of the isolates to standard antibiotics were high.

The findings of our study may be useful for healthcare authorities to plan interventions that can assist in optimizing the management of bacteriuria and UTI in the city of Daloa.

Contributions of authors:

GDA and ADM conceived the study idea; GDA, NNP and MS were involved in the study design; GDA, NNP, MS, GN and ADM were involved in analysis and interpretation of data; GDA and NNP produced the manuscript draft; and GN and ADM critically reviewed the manuscript for intellectual content. All authors read and approved the final manuscript submitted.

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No funding was received for the study.

Conflict of interest:

Authors declare no conflict of interest.

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