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Cotrimoxazole (ctx) Resistance in Common Bacterial Isolates from Patients with Pneumonia and Meningitis in Harare, Zimbabwe

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Research Article

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ABSTRACT

Introduction: Pneumonia and meningitis are important causes of morbidity and mortality in the immunocompromised patients. Resistance to cotrimoxazole (CTX) and other antimicrobial agents used for empirical treatment regimens is an important problem for both microbiologists and clinicians. CTX is the drug currently in use as a prophylaxis for patients on antiretroviral therapy. There is need to assess its efficacy and monitor any emergence of resistance by bacterial pathogens which may cause morbidity and mortality in the immunocompromised patients.

Methodology: A laboratory based cross-sectional study was used on a sample 200 bacterial isolates isolated from patients with pneumonia and meningitis was carried out. Pathogenic bacteria isolated from sputum and CSF samples of pneumonia and meningitis patients at five study sites were collected for culture and antimicrobial sensitivity testing during the period January 2014 to April 2014. The efficacy of cotrimoxazole and other antimicrobial drugs was calculated in percentage, per bacterial genus level. Tables were used to show the results for antibiotic susceptibility patterns. Graphs were used to show the minimum inhibitory concentration results.

Results: Pathogenic bacteria isolated from sputum, pleural effusions and bronchial washings of patients with pneumonia showed a high level of resistance to CTX. Generally, ciprofloxacin, gentamicin and ceftazidime were the most effective drugs against pathogenic bacteria isolated from patients with pneumonia. There was a high diversity of pathogenic bacteria isolated from CSF. S. pneumoniae was the most common organism isolated from CSF contributing 40% of the total. S. agalactiae was the second most common organism isolated from CSF contributing 17.5% of the total. All the isolates of S. agalactiae, H. influenzae, and Group D Streptococcus and S. pneumoniae were resistant to CTX. Tetracycline, doxycycline, chloramphenicol, ceftazidime, erythromycin and clindamycin were the most effective drugs against S. pneumoniae.

Conclusion and Recommendations: Pathogenic bacteria isolated from patients with pneumonia and meningitis showed a high level of resistance to cotrimoxazole. Results of this study suggest that use of CTX as a prophylactic drug for patients with HIV may not be effective for prevention of bacterial pneumonia and meningitis. The low number of organisms collected from patients with meningitis was mainly due to the fact that most cases of meningitis were due to Cryptococcus neoformans infection. There is need to carry out a larger study which encompasses both bacterial and fungal meningitis.

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INTRODUCTION

The increasing prevalence of HIV has led to an increase in respiratory tract infections and meningitis in immunocompromised patients [1,2]. In resource-constrained areas, prophylactic use of cotrimoxazole (CTX) is recommended for HIV-infected persons with CD4 counts <350 cells/mm^3 [1]. CTX is a combination of trimethoprim and sulfamethoxazole. CTX is widely used against Gram-positive bacteria e.g. *Streptococcus pneumoniae*, *S. aureus*, Gram-negative bacteria e.g. *Escherichia coli, K. pneumoniae* and non-typhoid Salmonella, protozoa e.g. *Toxoplasma gondii* and *Plasmodium falciparum* and fungi e.g. *Pneumocystis jirovecii* [1-3].

Most studies have revealed *Streptococcus* species to be the most common aetiological agents of bacterial meningitis and pneumonia. The genus *Streptococcus* is a member of the Streptococcaceae family [4]. *Streptococcus* species are Gram positive cocci growing in chains of various lengths, diplococci and as tetrads [4]. They are widely distributed in nature and can be found as normal flora of the human upper respiratory tract [4-6]. Common sites of colonization in healthy humans are the respiratory tract and gastro-intestinal tract [4-6]. *S.pneumoniae* has emerged as an important cause of all bacterial pneumonia contributing to 80% of the cases [4-6]. The prevalence and CTX susceptibility of *Streptococcus pneumoniae* isolated from sputum of 100 HIV-positive patients attending the Nigeria Institute of Medical Research clinic was investigated [7]. Eleven of the sputum specimens grew *Streptococcus pneumoniae* [7]. Antimicrobial susceptibility test showed that all the isolates were sensitive to augmentin, amoxicillin, chloramphenicol and erythromycin but were resistant to CTX [7]. In a non-randomised sub analysis of a trial study that was designed to measure the effectiveness of intravenous immunoglobin therapy in HIV-infected children, The National Institute of Child Health and Human Development Intravenous Study Group in 1991 demonstrated evidence that suggests that CTX prophylaxis might reduce infections caused by bacteria in HIV-infected people [8]. Clinical trials carried out by Anglaret et al. in 1999 in Cote d'Ivore showed that CTX prophylaxis reduced mortality in HIV-infected African adults with pulmonary tuberculosis and lowered hospital admission rates in adults with high CD4 cell counts without tuberculosis [9]. Chintu et al. in 2004 raised concerns that CTX prophylaxis may not work in areas with high levels of bacterial resistance to CTX [10].

Bacterial aetiology of pneumonia and meningitis

Acute bacterial meningitis is a common complication of HIV infection. HIV-positive patients are at increased risk for pneumococcal infection [11]. However, pneumonia is the most common manifestation of this predisposition [11]. *Streptococcus pneumoniae* and *Haemophilus influenzae* have been implicated as the main bacterial causes of pneumonia, with some severe cases caused by *Staphylococcus aureus* and *Klebsiella pneumoniae* also [12]. In South Africa, a prospective investigation on 250 children hospitalized with pneumonia who were known or clinically suspected to be HIV-positive was done with 151 children (60.4%) who were HIV-infected and *S. pneumoniae* was isolated 5% while *S. aureus* was isolated in 2% of these children [13]. In a study in Kenya, *Streptococcus pneumoniae* was the most common causative agent of pneumonia, being found in 46% cases and *Mycobacterium tuberculosis* was found in 9% [14]. In the United States of America, *Legionella pneumophila* was found in 2-25% of adults hospitalized for pneumonia [15]. *Mycoplasma pneumoniae* has also been implicated as an aetiological agent causing pneumonia [16]. *Pseudomonas aeruginosa* is an opportunistic pathogen which rarely causes pulmonary disease in normal hosts but which is an important cause of acute pneumonia in immunocompromised patients, including neonates.

Group B streptococcal meningitis was identified in 1.0% of HIV-infected American children diagnosed at the University of Maryland and the New York University Medical Center, with infection occurring beyond the usual age of onset in these children [17]. Marra et al. suggested that HIV infected patients are also at slightly increased risk for invasive infections with Neisseria meningitidis compared to the general population [18]. Pyogenic meningitis due to Methicillin Resistant S. aureus (MRSA) has rarely been reported. One case of MRSA pyogenic meningitis in an adult has been reported in which also the MRSA meningitis was spontaneous [19].

Pathogenesis of bacterial pneumonia and meningitis

Bacterial meningitis remains a disease with associated high morbidity and mortality rates despite the availability of effective bactericidal antimicrobial therapy. Most cases of bacterial meningitis begin with host acquisition of a pathogenic organism through nasopharyngeal colonization followed by systemic invasion and development of bacteraemia. Bacterial encapsulation contributes to this bacteraemia by inhibiting neutrophil phagocytosis and resisting classic complement-mediated bactericidal activity. Central nervous system invasion then occurs, although the exact site of bacterial traversal into the central nervous system is unknown [20]. By production and release of virulence factors into and stimulation of formation of inflammatory cytokines within the central nervous system, meningeal pathogens increase permeability of the blood-brain barrier, thus allowing protein and neutrophils to move into the subarachnoid space [20]. There is then an intense subarachnoid space inflammatory response, which leads to many of the pathophysiologic consequences of bacterial meningitis, including cerebral oedema and increased intracranial pressure [20]. Severe meningitis from encapsulated organisms such as Streptococcus pneumoniae, *N meningitides and Haemophilus influenzae* could result from a lack of activation of B cells by capsular antigens in patients with AIDS [21,22]. Investigations of B cell function in patients with AIDS have shown significantly lower antibody levels to polysaccharide and protein antigens after immunization with pneumococcal polysaccharide and protein antigens [21].

Pneumonia is broadly defined as any infection of the lungs [23,24]. Pneumonia can be caused by a range of infectious agents such as bacteria, viruses, fungi and parasites, with approximately 50% of all pneumonias being bacterial in origin [24,25]. Bacterial

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encounter may occur in various ways such as exposure to infectious respiratory droplets or the introduction of medical instruments into the host [30]. Most typical bacterial pneumonias are the result of initial colonization of the nasopharynx followed by aspiration or inhalation of organisms [23,24]. The alveolar macrophages constitute the first critical cellular defense. They can non-specifically phagocytose pathogens and may eliminate bacteria with no substantial inflammation [25]. However, a large bacterial load with high virulence evokes a protective inflammatory response [25,26]. Activation of the complement and coagulation cascades and the release of chemical mediators, such as leukotrienes, prostaglandins and histamine, contribute to increased blood flow to the infected site and increased capillary permeability [26]. A protein-rich inflammatory exudate containing leukocytes, antibodies and complement accumulate in the alveolar spaces leading to consolidation and impaired gaseous exchange [25,26]. Macrophages also produce proinflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL- 6) and tumor necrosis factor, which induce local inflammatory signs, fever and triggers catabolic responses [25-26].

Susceptibility of pathogens to cotrimoxazole and other antimicrobial agents

CTX is a combination of two synthetic drugs, Trimethoprim and Sulphamethoxazole [27-29]. TMP is an antifolate and Sulphamethoxazole is a sulphonamide [29]. Both drugs reduce the ability of some bacteria to utilize folic acid for growing. Sulphamethoxazole disrupts the production of dihydrofolic acid while trimethoprim disrupts the production of tetrahydrofolic acid [27,28]. Dihydrofolic acid and tetrahydrofolic acid are forms of folic acid that bacteria and human cells use for producing proteins [27,28]. TMP inhibits production of tetrahydrofolic acid by inhibiting the enzyme responsible for making tetrahydrofolic acid from dihydrofolic acid [29]. By combining both drugs, two important steps required in the production of bacterial proteins are interrupted, and the combination is more effective than either drug alone [27]. Usually bacteria become resistant to CTX by production of metabolic pathways that bypass the site of antimicrobial action [29]. The synergistic effects of CTX can be compromised by resistance to either single component or in some cases resistance to both components [28].

Nyasulu et al. (2012) carried out a systematic review of published data in South Africa on the antimicrobial susceptibility patterns of common bacterial respiratory pathogens. The review showed that 29% of *S aureus* isolates were resistant to cloxacillin and 38% to erythromycin. For *K pneumoniae*, resistance to ciprofloxacin was 35% and to ampicillin 99%; and for *P aeruginosa*, the mean resistance to ciprofloxacin was 43% and to amikacin 35% [29]. Gill et al. (2007), on a study in Zambia, measured the microbiological consequences of implementing WHO guidelines of CTX prophylaxis using pneumococcus [30]. Eighty per cent of the isolates were found to be resistant to CTX [30].

A study carried out in Kenya on antimicrobial susceptibilities of bacterial isolates isolated from adults with community acquired pneumonia showed that prevalence of resistance to penicillin and other commonly used antibiotics among pneumococci is high and the large number of multi-resistant strains among *H. influenzae* is a cause for concern [31]. A total of 277 *S. pneumoniae* and 58 *H. influenzae* were obtained from 536 adults examined in the period January 1998 to December 1999 [31]. Of the 277 *S. pneumoniae*, only 56.7% were susceptible to penicillin and 7.6% of strains were resistant to two or more antimicrobial agents [31]. Of the 58 *H. influenzae* strains, 91.4% were sensitive to ampicillin, with 6.8% resistant to two or more antimicrobial agents [31].

A study in Ethiopia to determine susceptibility patterns of bacterial aetiological agents of meningitis showed that *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Nessieria meningitidis* were the most common pathogens isolated from CSF. *Streptococcus pneumoniae* was sensitive to ceftriaxone, ciprofloxacin, chloramphenicol, erythromycin and rifampicin and showed low level of resistance (<60%) to penicillin, tetracycline and trimethoprim-sulphamethoxazole [32]. *Haemophilus influenzae* and *Nessieria meningitidis* showed high level of resistance (>80%) to tetracycline and trimethoprim-sulphamethoxazole, intermediate level of resistance (60-80%) to ampicilin and low level of resistance (<60%) to ceftriaxone, ciprofloxacin, gentamicin, chloramphenicol and rifampicin [32].

A study carried out in Ethiopia on meningitis revealed that the most commonly isolated bacteria were *Neisseria meningitidis* 10 (45.5%) and *Streptococcus pneumoniae* 7 (31.8%) [33]. The Ethiopian study showed that among Gram positive organisms, *S. pneumoniae* showed a high level of drug resistance against chloramphenicol 4 (57%), tetracycline 3 (43%), CTX 3 (43%), ampicillin 3 (43%), and gentamicin 1 (14%) [33]. *N. meningitidis* was shown to be resistant to CTX 5 (50%), chloramphenicol 3 (30%), gentamicin 3 (30%) and ampicillin 2 (20%) [33]. The single isolate of Proteus species was found to be resistant to CTX and tetracycline. *E. coli* was found to be resistant to all antibiotics except for gentamicin and ciprofloxacin [33]. Multiple drug resistance was observed in 50% of the isolates [14]. No organism showed resistance to ciprofloxacin [33].

A study carried out in Egypt on antimicrobial susceptibilities of *S. pneumoniae* isolates isolated from patients with meningitis showed that resistance to penicillin may be increasing among *S. pneumoniae* strains causing meningitis in Egypt [34]. Forty-nine percent of all isolates were found to be resistant to penicillin, 6% resistant to ceftriaxone, 52% resistant to tetracycline, 60% resistant to CTX, 11% resistant to erythromycin and 9% resistant to chloramphenicol [34].

MATERIALS

Materials which were used in this study are listed on appendix 1.

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METHODS

Study site

The study was conducted at five sites; two tertiary hospitals and three private laboratories in Harare Zimbabwe where routine clinical samples were received from local clinics and hospitals as well as referral district and provincial hospitals and processed.

Sample size

A total of 200 bacterial isolates i.e. 160 and 40 from pneumonia and meningitis respectively were used in this study. The calculation of the sample size is found on appendix 2.

Ethical considerations

Permission to carry out the study was sought from the Joint Parirenyatwa- UZ Research Ethics Committee and the bacterial isolates were taken after permission was sought from the managers and microbiology head of department at respective laboratories.

Collection of bacterial isolates

Bacterial isolates were transported to the University of Zimbabwe, Department of Medical Laboratory Sciences laboratory for processing, on Mueller Hinton agar plates from the respective laboratories. *Haemophilus influenzae* and *S. pneumoniae* were transported on Chocolate Agar plates. The bacterial species, the sample from which isolated, and the laboratory where the sample was processed was recorded. Laboratory numbers were used to identify the bacterial isolates i.e from sputum (e.g. Bt 01, Bt 02) and from CSF (e.g. Bv 01, Bv 02).

Bacteria from sputum

Gram negative bacteria and *S. aureus* were cultured on MacConkey agar plates and incubated aerobically for 24 hours at 37ºC. Single colonies of respective organisms were sub cultured on new MacConkey agar plates to obtain pure cultures. *S.aureus* was also sub cultured on Blood Agar plates to determine the type of haemolysis. *S. pneumoniae* was cultured on Chocolate agar and an optochin disc added to the plate and incubated in a candle jar for 24 hours at 37ºC. *S. pneumoniae* was identified as optochin sensitive and α haemolytic and was purified on new Chocolate Agar plates.

Bacteria from CSF

S. aureus was cultured on Blood Agar and MacConkey agar and incubated aerobically for 24 hours at 37ºC. *S. pneumoniae* and *H. influenzae* were cultured on Chocolate agar and an optochin disc added to the plate inoculated with *S. pneumoniae*. *S. agalactiae* and Group D *Streptococcus* were cultured on Blood Agar plates and incubated in a candle jar for 24 hours at 37ºC. Single colonies were picked and sub cultured on respective plates to get pure cultures.

Identification of isolates

Isolates collected after they had been identified and serotyped at respective laboratories. The bacterial colonies were used to prepare smears which were Gram stained as described on appendix 3 and observed under the light microscope at x100 objective lens using immersion oil. Streptococcus species were identified as Gram-positive cocci in chains and for *S. pneumoniae* in pairs. *Staphylococcus* species i.e. Gram-positive cocci in clusters were identified using the biochemical tests shown on (**Table 5**) found on **Appendix 4**. The tests used in the identification of Streptococcus species are shown on (**Table 6**) found on **Appendix 4**. Lactose fermenting rods were identified using biochemical tests shown on (**Table 7**) found on **Appendix 4**. The procedures for biochemical tests are described on **Appendix 5**. *H. influenzae* was identified as Gram-negative rods, requiring both X and V factors for growth and not growing on X of V factors only and satellitism positive. *Acinetobacter baumannii* was identified as Gram-negative coccobacilli, non-motile, oxidase negative and confirmed by the analytical profile index (API). Pantoea species were confirmed by API with a distinguishing feature of failure to utilize the amino acids lysine, arginine, and ornithine that sets it apart from other Enterobacteriaceae.

Antimicrobial Susceptibility Testing and the E test

The modified Kirby Bauer disk diffusion test, which conforms to the recommended standards of the Clinical and Laboratory Standards Institute (CLSI), was used in this study. Three colonies of each pure bacterial isolate were emulsified in 3ml of sterile normal saline in a bijou bottle and the inoculum density compared to the barium chloride standard / 0.5 McFarland solution. A dry sterile swab was dipped into the bacterial emulsion, rotated around the neck to remove excess bacteria and inoculated on Mueller Hinton Agar using the lawn technique and allowed to dry before adding drugs and incubated at 37 °C for 24 hours. Up to eight commercially prepared, fixed concentrations, paper antibiotic discs were placed on the inoculated agar surface. For *Haemophilus influenzae* and *S.pneumoniae*, a loopful of the organism was inoculated on Chocolate agar using the lawn technique and plates incubated in a candle jar, both for 24 hours at 37 °C. The following antibiotic discs were added in less than 15 minutes after inoculation of the plates with bacteria, spaced enough to avoid overlapping of zones of inhibition : Cotrimoxazole (25 µg) , Trimethoprim (25 µg), Ceftazidime (30 µg), Cefuroxime (30 µg), Tetracycline (30 µg), Ampicillin (25 µg) , Amikacin (10 µg), Kanamycin (30 µg),

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Streptomycin (10 µg), Gentamicin (10 µg), Doxycycline (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Penicillin G (10 µg), Fusidic acid (5 µg), Oxacillin (1 µg), Erythromycin (15 µg), and Clindamycin (2 µg) . The zone of inhibition diameters was measured in millimetres and compared with recorded critical diameters of respective antibiotics on a zone size as per CLSI interpretative table, to determine resistance or susceptibility. To ensure quality, reference strains *E.coli* ATCC 25922 and *S.aureus* ATCC25923 control strains were used to control media and antibiotic discs as well as E test strips whenever a new batch of media and antibiotics was used. A cotrimoxazole E strip was placed on another Mueller Hinton plate inoculated using the lawn technique. The plates were incubated aerobically at 37 °C. The drug concentration where the ellipse of bacterial growth intersects with the MIC scale on the E test strip was recorded as the MIC. *E.coli* ATCC 25922 was used to control media, antibiotic discs as well as E test strips weekly and whenever a new batch of media or antibiotic discs was used.

RESULTS

Of the 160 bacterial isolates isolated from patients with pneumonia, 145 were from sputum samples, 13 from pleural effusions and 2 from bronchial washings. The distribution of pathogens from patients with pneumonia is shown on **Table 1**.

Table 1. Distribution of pathogenic bacteria isolated from patients with pneumonia

a. Sputum

Bacterial pathogen	Number of isolates (n=145)	Percentage (%)
P. aeruginosa	46	31.7
K. pneumoniae	43	29.7
S.aureus	36	24.8
E. coli	10	6.9
H. influenzae	3	2.1
A. baummannii	3	2.1
Proteus species	2	1.4
Pantoea species	1	0.7
M. catarrhalis	1	0.7

b. Pleural effusion

Bacterial pathogen	Number of isolates (n=13)	Percentage (%)
K. pneumoniae	7	43.8
S. aureus	3	23.1
P. aeruginosa	3	23.1

c. Bronchial washing

Bacterial pathogen	Number of isolates (n=2)	Percentage (%)
K. pneumoniae	1	50.0
A. baummannii	1	50.0

K. pneumoniae was the most common organism isolated from patients with pneumonia contributing 32% of the isolates. *P. aeruginosa* was the second most common organism isolated from patients with pneumonia contributing 31% of the isolates. Single isolates of Pantoea species and *M. catarrhalis* were collected. Organisms isolated from pleural effusions contributed 8% of the total bacterial isolates with *K. pneumoniae* being the most common organism isolated from this specimen type. Single isolates of *K. pneumoniae* and *A. baummannii* were collected from bronchial washings.

The susceptibility patterns of the organisms isolated from patients with pneumonia are shown on Table 2. Pathogenic bacteria isolated from sputum, pleural effusions and bronchial washings of patients with pneumonia showed a high level of resistance to CTX. Generally, ciprofloxacin, gentamicin and ceftazidime were the most effective drugs against pathogenic bacteria isolated from patients with pneumonia. All the isolates of *P. aeruginosa*, *Acinetobacter*, *H. influenzae* were resistant to CTX. All the three *H. influenzae* isolates were resistant to both ampicillin and chloramphenicol. No *H. influenzae* strain was resistant to ciprofloxacin. Thirteen (27%) of *P. aeruginosa* isolates were multi-drug resistant showing resistance to all the 12 antibiotics used. Fifteen (39%) of *S. aureus* isolates were susceptible to CTX with eight (53%) of the isolates showing MIC's greater than 0.25mcg/ ml as shown on Figure 1. Fourteen (36%) of *S. aureus* isolates were Methicillin Resistant *S. aureus* (MRSA) based on their resistance to oxacillin and were showing multi-drug resistance with doxycycline and streptomycin being the most effective antibiotics for these strains. Sixteen (31%) of *K. pneumoniae* isolates were sensitive to CTX. The distribution of cotrimoxazole MIC's in the 51 *K. pneumoniae* isolates is shown in Figure 2. Ceftazidime, ciprofloxacin, chloramphenicol and amikacin were the most effective drugs against *K. pneumoniae*. Three (30%) of the ten isolates of *E. coli* were sensitive to CTX with MIC's of 0.094, 0.25 and 0.38 mcg/ml. However, all ten isolates of *E. coli* were sensitive to ceftazidime and gentamicin. Both Proteus isolates were resistant to CTX. The single isolates of *M. catarrhalis* and *Pantoea* species were both resistant to CTX. The *Pantoea* species was multi-drug resistant showing resistance to trimethoprim, streptomycin, ampicillin, tetracycline, doxycycline, chloramphenicol, kanamycin, ciprofloxacin, gen-

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tamicin, and ceftazidime. *M. catarrhalis* was resistant to cotrimoxazole, trimethoprim, ampicillin and tetracycline. *Acinetobacter* species showed multidrug resistance to CTX, trimethoprim, chloramphenicol, tetracycline, ampicillin and amikacin with all four isolates being sensitive to gentamicin.

Table 2 Antimicrobial susceptibilities of bacteria isolated from patients with pneumonia.

	***K. pneumoniae* (n=51)**						***P. aeruginosa* (n=49)**						***S. aureus* (n=39)**					
	S		**I**		**R**		**S**		**I**		**R**		**S**		**I**		**R**	
	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)
Cotrimoxazole	16	(31.4)	0	(0)	35	(68.6)	0	(0)	0	(0)	49	(100)	15	(38.5)	0	(0)	24	(61.5)
Trimethoprim	10	(19.6)	1	(2.0)	40	(78.4)	0	(0)	0	(0)	49	(100)	15	(38.5)	0	(0)	24	(61.5)
Streptomycin	15	(29.4)	0	(0)	36	(70.6)	0	(0)	0	(0)	49	(100)	25	(64.1)	1	(2.6)	13	(33.3)
Gentamicin	25	(47.0)	2	(3.0)	24	(49.0)	15	(30.6)	4	(8.2)	30	(61.2)	30	(76.9)	0	(0)	9	(23.1)
Amikacin	25	(49.0)	0	(0)	26	(51.0)	0	(0)	0	(0)	49	(100)	10	(25.6)	0	(0)	29	(74.4)
Kanamycin	21	(41.2)	0	(0)	30	(58.8)	10	(20.4)	0	(0)	39	(79.6)	26	(66.7)	0	(0)	13	(33.3)
Doxycycline	10	(19.6)	5	(9.8)	36	(70.6)	0	(0)	8	(16.3)	41	(83.7)	26	(66.7)	0	(0)	13	(33.3)
Tetracycline	0	(0)	10	(19.6)	41	(80.4)	0	(0)	0	(0)	49	(100)	8	(20.5)	1	(2.6)	23	(76.9)
Chloramphenicol	30	(58.8)	1	(2.0)	20	(39.2)	0	(0)	2	(4.1)	47	(95.9)	29	(74.4)	0	(0)	10	(25.6)
Ciprofloxacin	36	(70.6)	0	(0)	15	(29.4)	19	(38.8)	0	(0)	30	(61.2)	25	(64.1)	1	(2.6)	13	(33.3)
Ceftazidime	40	(78.4)	0	(0)	11	(21.6)	14	(28.6)	6	(12.2)	29	(59.2)	29	(74.4)	0	(0)	10	(25.6)
Ampicillin	0	(0)	0	(0)	51	(100)	0	(0)	0	(0)	49	(100)	3	(7.7)	0	(0)	36	(92.3)
Penicillin G													0	(0)	0	(0)	39	(100)
Fusic acid													24	(61.5)	6	(15.4)	9	(23.1)
Oxacillin													14	(35.9)	0	(0)	25	(64.1)
Erythromycin													29	(74.4)	0	(0)	10	(25.6)
Clindamycin													30	(76.9)	0	(0)	9	(23.1)

Key: S= sensitive, I= intermediate, R= resistant

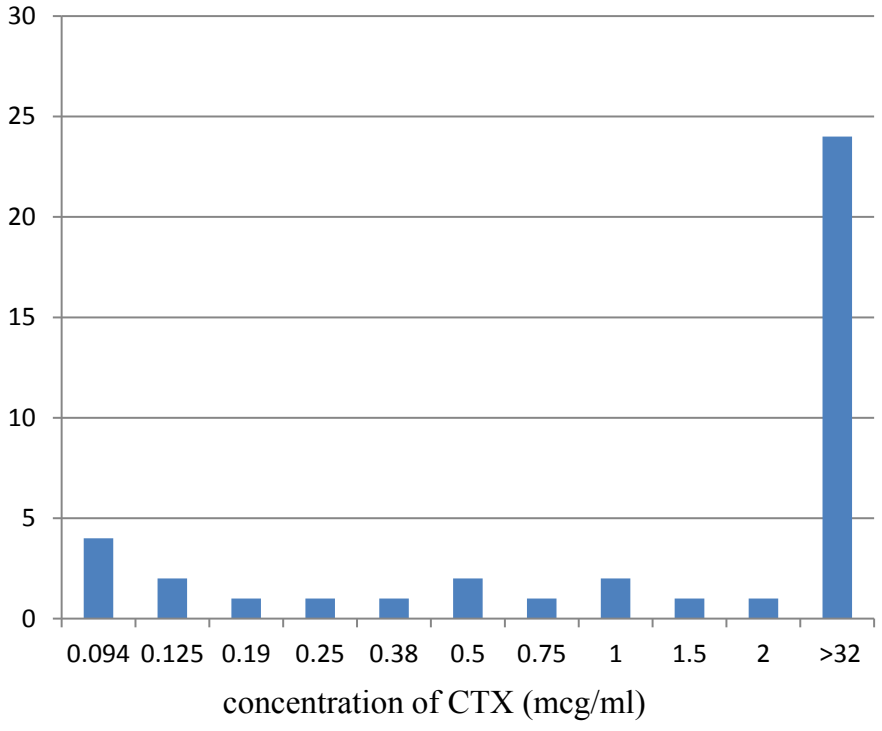

Figure 1. Distribution of cotrimoxazole MICs in 39 S. aureus species.

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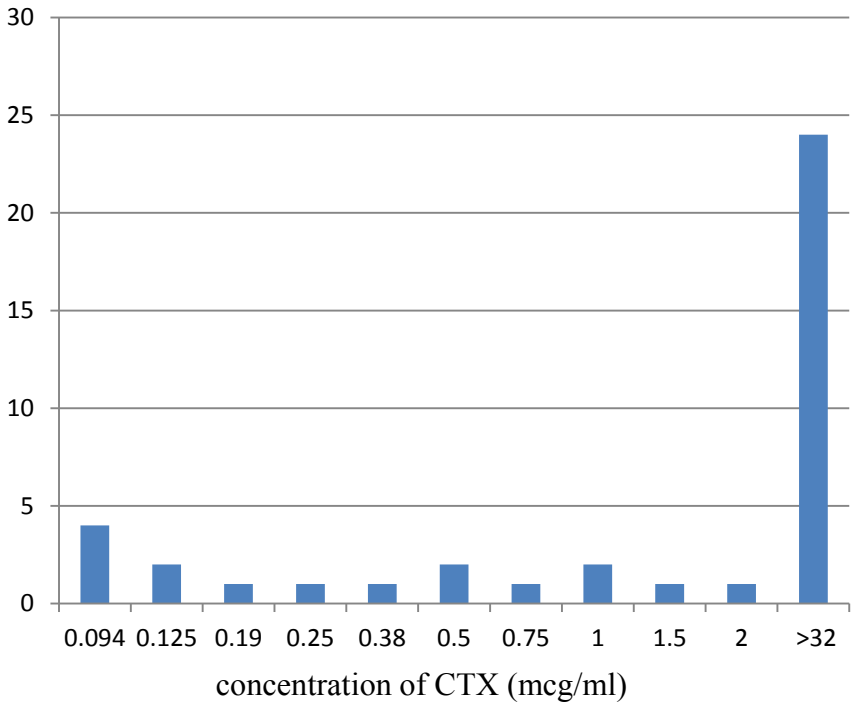

Figure 2. Distribution of cotrimoxazole MICs in 51 K. pneumoniae species.

The distribution of pathogens from patients with meningitis is shown on **Table 3**.

Table 3. Distribution of pathogenic bacteria isolated from patients with meningitis.

Bacterial pathogen	Number of isolates (n=40)	Percentage (%)
S. pneumoniae	16	40.0
S. agalactiae	7	17.5
S. aureus	4	10.0
S. epidermidis	3	7.5
H. influenzae	3	7.5
Group D Streptococcus	3	7.5
E. coli	2	5.0
Citrobacter species	1	2.5
S. pyogenes	1	2.5

There was a high diversity of pathogenic bacteria isolated from CSF. *S. pneumoniae* was the most common organism isolated from CSF contributing 40% of the total. *S. agalactiae* was the second most common organism isolated from CSF contributing 17.5% of the total. *S. epidermidis*, *H. influenzae* and Group D *Streptococcus* each contributed 7.5%. The least common organism was *S. pyogenes* and *Citrobacter* species each contributing 2.5% of the total.

The susceptibility patterns of the organisms isolated from CSF are shown on **Table 4.** All the isolates of *S. agalactiae*, *H. influenzae*, Group D *Streptococcus* and *S. pneumoniae* were resistant to CTX. Tetracycline, doxycycline, chloramphenicol, ceftazidime, erythromycin and clindamycin were the most effective drugs against *S. pneumoniae*. The distribution of cotrimoxazole MIC's in the seven Staphylococcus species. Five (71.4%) of the *Staphylococcus* species isolated from CSF were sensitive to CTX with two (40%) of them having MIC's greater than 1 mcg/ml, that is in the upper limit of the susceptibility range, towards the lower limit of resistance. The remaining 28.6% CTX resistant *Staphylococcus* isolates showed very high level of resistance to the drug with MIC's greater than 32 mcg/ ml. One (14.3%) of *S. aureus* isolates from CSF was Methicillin Resistant *S. aureus* (MRSA) based on its resistance to oxacillin and showed multi-drug resistance with susceptibility to doxycycline only. Both isolates of *E. coli* were susceptible to CTX with MIC's of 0.25 and 0.38 mcg/ml. Two (66.7%) of the Group D *Streptococcus* isolates were susceptible to chloramphenicol, ceftazidime and erythromycin. All the three *H. influenzae* isolates were resistant to both ampicillin and chloramphenicol. No *H. influenzae* strain was resistant to ciprofloxacin. *S. pyogenes* was susceptible to erythromycin, clindamycin and ampicillin. The single Citrobacter species was multi-drug resistant showing sensitivity to ceftazidime only.

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Table 4: Antimicrobial susceptibilities of bacteria isolated from patients with meningitis

	S. pneumoniae (n=16)						*S. agalactiae* (n=7)						Staphylococcus species (n=7)					
	S		I		R		S		I		R		S		I		R	
	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)
Cotrimoxazole	0	(0)	0	(0)	16	(100)	0	(0)	0	(0)	7	(100)	5	(71.4)	0	(0)	2	(28.6)
Trimethoprim	0	(0)	0	(0)	16	(100)	0	(0)	0	(0)	7	(100)	5	(71.4)	0	(0)	2	(28.6)
Streptomycin	10	(62.5)	0	(0)	6	(37.5)	4	(57.1)	2	(28.6)	1	(14.3)	7	(100)	0	(0)	0	(0)
Gentamicin	10	(62.5)	0	(0)	6	(37.5)	3	(42.9)	0	(0)	4	(57.1)	7	(100)	0	(0)	0	(0)
Amikacin	0	(0)	0	(0)	16	(100)	2	(16,7)	0	(0)	5	(71.4)	7	(100)	0	(0)	0	(0)
Kanamycin	0	(0)	0	(0)	16	(100)	5	(71.4)	0	(0)	2	(28.6)	5	(71.4)	0	(0)	2	(28.6)
Doxycycline	16	(100)	0	(0)	0	(0)	1	(14.3)	2	(28.6)	4	(57.1)	7	(100)	0	(0)	0	(0)
Tetracycline	16	(100)	0	(0)	0	(0)	1	(14.3)	2	(28.6)	4	(57.1)	5	(71.4)	0	(0)	2	(28.6)
Chloramphenicol	16	(100)	0	(0)	0	(0)	4	(57.1)	2	(28.6)	1	(14.3)	7	(100)	0	(0)	0	(0)
Ciprofloxacin	10	(62.5)	0	(0)	6	(37.5)	3	(42.9)	0	(0)	4	(57.1)	7	(100)	0	(0)	0	(0)
Ceftazidime	16	(100)	0	(0)	0	(0)	5	(71.4)	0	(0)	2	(28.6)	7	(100)	0	(0)	0	(0)
Ampicillin	3	(18.7)	0	(0)	13	(81.3)	2	(28.6)	0	(0)	5	(71.4)	2	(28.6)	0	(0)	5	(71.4)
Penicillin G	6	(37.5)	0	(0)	10	(62.5)	4	(57.1)	2	(28.6)	1	(14.3)	0	(0)	0	(0)	7	(100)
Fusic acid	0	(0)	0	(0)	16	(100)	1	(14.3)	2	(28.6)	4	(57.1)	5	(71.4)	0	(0)	2	(28.6)
Oxacillin	0	(0)	0	(0)	16	(100)	3	42.9)	0	(0)	4	(57.1)	6	(85.7)	0	(0)	1	(14.3)
Erythromycin	16	(100)	0	(0)	0	(0)	5	(71.4)	0	(0)	2	(28.6)	7	(100)	0	(0)	0	(0)
Clindamycin	16	(100)	0	(0)	0	(0)	3	(42.9)	0	(0)	4	(57.1)	7	(100)	0	(0)	0	(0)

Key: S= sensitive, I= intermediate, R= resistant.

DISCUSSION

Pathogenic bacteria isolated from patients with pneumonia and meningitis showed a high level of resistance to cotrimoxazole. In Zimbabwe, Ministry of Health and Child Welfare (2011) in The Essential drug lists and standard treatment guidelines for Zimbabwe (EDLIZ) 6th edition, recommends the use of CTX prophylaxis in HIV infected patients with an average adult dose of 960 mg taken every day orally for life or until CD4>200 cells/mm3 for 3 months with ARVs [36]. Results of this study suggest that use of CTX as a prophylactic drug for patients with HIV may not be effective for prevention of bacterial pneumonia and meningitis.

The low number of organisms collected from patients with meningitis was mainly due to the fact that most cases of meningitis are due to Cryptococcus neoformans infection. Cryptococcus neoformans was in the rejection criteria in this study. There is need to carry out a larger study which encompasses both bacterial and fungal meningitis.

The Essential Drug List and Standard Treatment Guidelines for Zimbabwe recommend gentamicin and chloramphenicol for use in treating pneumonia caused by Gram negative bacteria [35-36]. In this present study 121 (75.6%) of the 160 bacterial isolates isolated from patients with pneumonia were Gram-negative bacteria. Of the 121, 50 (41%) were sensitive to both chloramphenicol and gentamicin. This shows that these drugs are still effective against Gram negative bacteria causing pneumonia. However, of the 121, Gram negative bacteria 57 (47%) were sensitive to both the quinolone ciprofloxacin and the cephalosporin ceftazidime. This finding suggests that ciprofloxacin and ceftazidime can be used in treating pneumonia caused by Gram negative bacteria in addition to gentamicin and chloramphenicol.

Thirty (77.0%) of *Staphylococcus* species isolated from patients with pneumonia were susceptible to clindamycin. This finding shows that the drug is still effective in treating pneumonia caused by *Staphylococcus* species and supports the recommendation in EDLIZ to use this antibiotic in *Staphylococcal pneumonia*. However, results of the present study showed that gentamicin may be equally effective in treating *Staphylococcal pneumonia*. In collection of samples, it was observed that most cases of suspected hospital acquired pneumonia were caused by *Pseudomonas aeruginosa*. 15 (30.6%) of *Pseudomonas aeruginosa* isolates were sensitive to gentamicin, the drug recommended in EDLIZ for treatment of hospital acquired pneumonia [35-36]. However, 19 (38.8%) of the *Pseudomonas aeruginosa* isolates were sensitive to ciprofloxacin. Furthermore, the most commonly isolated organisms from patients with pneumonia were *Pseudomonas aeruginosa*, *K. pneumoniae* and *S. aureus* contributing 139 (86.9%) of the

160 organisms collected. Of these 139, 70 (50.4%) and 80 (57.6%) were sensitive to gentamicin and ciprofloxacin respectively. This finding suggests that ciprofloxacin can be used in treating hospital acquired pneumonia in addition to gentamicin.

All the 39 *Staphylococcus* isolates isolated from patients with pneumonia were resistant to penicillin G. This finding suggests that this drug may no longer be useful in treating *Staphylococcal pneumonia*. However, *Staphylococcus* species were highly susceptible to erythromycin, clindamycin and doxycycline. This finding supports the recommendation in EDLIZ to use these antibiotics in treating *Staphylococcal pneumonia* and meningitis [35]. Gentamicin proved to be effective also against *Staphylococcus* aureus isolated from pneumonia and meningitis with susceptibilities of 77% and 100% respectively.

Seven (17.5%) of the 40 bacterial isolates from CSF were sensitive to CTX and two (28.5%) of these isolates had MIC's greater than 1 mcg/ml, that is in the upper limit of the susceptibility range, towards the lower limit of resistance. Twenty-eight (17.5%) of the 160 bacterial isolates from patients with pneumonia were sensitive to CTX. These findings further support that cotrimoxazole prophylaxis in the prevention of pneumonia and meningitis is very low. Generally, from the trend observed, the resistance of all pathogenic bacteria causing pneumonia and meningitis to CTX was quite high.

Chloramphenicol recommended in EDLIZ for use in treating meningitis was proved to be still useful as results of the present study showed all the isolates of *Staphylococcus* and *S. pneumoniae* and 83.3% of *S. agalactiae* to be susceptible to the drug. However, all *Staphylococcus* species isolated from CSF were resistant to Penicillin G. Penicillin G is recommend in EDLIZ 2011 for use in bacterial meningitis, [36], may not be effective in treating *Staphylococcal meningitis*. Chloramphenicol may be the drug of choice in treating *Staphylococcal meningitis* as results of the present study showed all the isolates of *S. aureus* to be susceptible to the drug. The study also showed that *S. pneumoniae* isolates to be resistant to ciprofloxacin as 54.5% of the isolates were resistant to the drug. Ciprofloxacin is recommended in EDLIZ for chemoprophylaxis for close contacts in cases of meningococcal meningitis [35,36].

CONCLUSION

The study showed that most pathogenic bacteria causing pneumonia and meningitis are resistant to CTX. The results of the study show that ciprofloxacin and ceftazidime can be used in treating pneumonia caused by Gram negative bacteria in addition to gentamicin and chloramphenicol. The results of the study suggest that ciprofloxacin can be used in treating hospital acquired pneumonia in addition to gentamicin. The study also showed chloramphenicol to be still effective in treatment of bacterial meningitis.

CONFLICT OF INTEREST

The author does not have a commercial or other association that might pose a conflict of interest with any organization.

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