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Determination of Antibiotic Susceptibility Profile of some Enterobacteria Isolated from Respiratory Tract Infection Patients Attending some Tertiary Hospital in Kano, Northern Nigeria

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Abstract

Bacterial antibiotic resistance is a type of drug resistance whereby some sub-populations of bacterial species are able to survive after exposure to one or more antibiotics. The study was aimed to determine the antibiotic susceptibility profile of some enterobacteria from respiratory tract infection (RTIs) patients attending some tertiary Hospital in Kano, Northern Nigeria. A total of one thousand and ninety six (1096) isolates were collected. Isolates were subjected to Gram staining, motility test, biochemical characterization and further examined using Microgen TM Gram negative Identification A (Microgen GN ID A) system. Susceptibility of the isolates to some commonly used antibiotics was determined using the disc diffusion method. The result showed that various Enterobacteriaceae isolates confirmed were *Pantoae agglomerans* 250 (53.53%) being the most occurring followed by *Klebsiella spp* 160 (34.26%) and then *Escherichia coli* 57 (12.21%) respectively. The antibiotic resistance pattern of the isolates showed that the highest resistance level was recorded for Ampicillin (78%), followed by Amoxycillin (72%), Ceftazidime (42%). There is also a significant level of resistance to Sulfamethoxazole trimethoprim (36%), Ceftriaxone (28%) and chloramphenicol (24%). Lower resistance levels were observed against Gentamicin (8%) and Ciprofloxacin (10%). It is concluded that there is significant level of antibiotic resistant of some isolates from respiratory tract infection (RTIs) patients.

Keywords: Antibiotic resistant, Enterobacteria, Kano, Respiratory tract infection.

INTRODUCTION

Bacterial antibiotic resistance is a type of drug resistance whereby some sub-populations of bacterial species are able to survive after exposure to one or more antibiotics [1]. In other words, ―antibiotic resistance means the ability of a microorganism to withstand the effect of an antibiotic [2]. Several mechanisms have evolved in bacteria which confer them with antibiotic resistance to ensure their survival. These mechanisms can chemically modify the antibiotic, render it inactive through physical removal from the cell, or modify target site so that it is not recognized by the antibiotic. The most common mode is enzymatic inactivation. An existing cellular enzyme is modified to react with the antibiotic in such a way that it no longer affects the microorganism [3]. The worldwide spread of drug resistance among common respiratory pathogens, including Gram-negative pathogens are particularly worrisome because they are becoming resistant to nearly all the antibiotic drug options available, creating situations reminiscent of the pre-antibiotic era [4-6].

Respiratory tract infection is a public health concern in global scale. Respiratory tract is the part of human system that plays a vital role in breathing process. In human, the respiratory system can be sub-divided into upper respiratory tract and lower respiratory tract based on anatomical features [7]. Upper respiratory tract infections (URI or URTI) are illnesses caused by an acute infection which involves

the upper respiratory tract including the nose, sinuses, pharynx or larynx. Acute lower respiratory tract infections are a persistent and pervasive public health problem. They cause a greater burden of disease worldwide than human immunodeficiency virus infection, malaria, cancer, or heart attacks. The outcome of an acute lower respiratory tract infection depends on the virulence of the organism and the inflammatory response in the lung. Acute lower respiratory tract infections can be monomicrobial or polymicrobial, with organisms ranging in virulence from commensal to highly pathogenic [8].

Members of the bacterial family Enterobacteriaceae are found in the environment but also make up part of the normal microbiota of the intestine in humans and other animals. They are rod-shaped and stain Gram-negative, non-sporulating, facultative anaerobes that ferment different carbohydrates to obtain carbon [9]. They may grow as mucoid colonies when cultivated on agar plates, but only *Klebsiella* spp. are truly encapsulated [10]. The Enterobacteriaceae can be divided in 51 genera [11] and the number of species is continuously increasing. Members of the Enterobacteriaceae can cause many different kinds of infections. Urinary tract infections (UTIs) are the most common, followed by pneumonia, respiratory infection, wound infections and infections of the bloodstream and central nervous system. Some genera are common causes of intestinal infections such as enteritis and diarrhea. They also make up an essential part of nosocomial infections, especially catheter related UTIs and ventilator associated pneumonia [9-13]. The study was aimed to determine the antibiotic susceptibility profile of some enterobacteria from respiratory tract infection patients attending some tertiary Hospital in Kano, Northern Nigeria

MATERIALS AND METHODS

Ethical Approval

Ethical clearance with the reference approval number - NHREC/17/03/2018 were obtained from the Kano State Ministry of Health based on research ethics committees of Murtala Muhammad Specialist Hospital (MMSH), Muhammad Abdullahi Wase Teaching Hospital (MAWTH), and Sir Muhammad Sunusi Specialist Hospital (SMSSH).

Sampling Sites

The samples were collected from Murtala Muhammad Specialist Hospital (MMSH), Muhammad Abdullahi Wase Teaching Hospital (MAWTH), and Sir Muhammad Sunusi Specialist Hospital (SMSSH), all in Kano metropolis. Kano State was located North-western part of Nigeria between latitude 11^0 58' and 12^0 01' North and longitude 8^0 29' and 8^0 33' East.

Collection of the isolates

A total of one thousand and ninety six (1096) isolates were collected from the Microbiology laboratory of all the 3 study sites i.e., Murtala Muhammad Specialist Hospital (MMSH), Muhammad Abdullahi Wase teaching Hospital (MAWTH) and Sir Muhammad Sunusi Specialist Hospital (SMSSH) all in Kano State, North Western Nigeria. The isolates were collected within the period of 9 months (May 2017 to February, 2018).

Table 1: Number and percentage of Isolates collected from each of the sampling site

Sampling site	Number of isolates	Percentage (%)
MMSH	498	45.44
MAWTH	373	34.03
SMSSH	225	20.53
Total	**1096**	**100**

Identification of the isolates

Isolates were subjected to Gram staining, motility test and biochemical characterization as described by Cheesbrough [14]. Isolates tested were examined further using Microgen TM Gram negative Identification A (Microgen GN ID A) system. The data obtained by the Microgen GN-ID A micro well strip was designed to generate a 4 digit octal code which was used to interpret the result by the Microgen Identification System Software.

Antibiotic Susceptibility Testing

Susceptibility of the isolates to some commonly used antibiotics was determined using the disc- diffusion method as recommended by Clinical Laboratory Institute Standards [15]. The bacterial isolates were cultured for 24 hours on Nutrient broth. They were suspended in 2 ml sterile normal saline and turbidity adjusted to match 0.5 McFarland Opacity Standard. Bacterial suspensions of 0.1 ml were dispensed on the surface of the Mueller-Hinton agar plate and spread evenly. The inoculum was allowed to dry for 5 min and antibiotic discs were dispensed on the surface of the media and incubated aerobically at 37^0C for 24 hours. Results were classified as susceptible or resistant, according to the approved clinical breakpoints [15].

RESULTS

Identification of the isolates

A total of one thousand and ninety six (1096) isolates were collected from the Microbiology laboratory of all the 3 study sites. The identity of these bacterial isolates was confirmed through Gram staining

reaction, motility and conventional biochemical tests. Out of these isolates collected, 301 (27.5%) were observed to be Gram positive while 732 (66.8%) were Gram negative, those isolates that yielded no growth were 63 respectively (5.7%) as presented in Table 1 below.

Table 1: Identification of the isolates collected from study sites

Gram's reaction	Number of isolates	Percentage occurrence (%)
Gram positive	301	27.5
Gram negative	732	66.8
No growth	63	5.7
Total	**1096**	**100**

Identification of Enterobacteria Isolates

The identified Gram negative Enterobacteriaceae isolates after purifying were later subjected to biochemical tests for further identification, confirmation and characterization of which Microgen GN A ID kit was used. Microgen identification results shows that various Enterobacteriaceae isolates confirmed were *Pantoae agglomerans, Klebsiella spp* and then *Escherichia coli*.

Table 2: Microgen GN A Identification results for the confirmed isolates

S/N	Lys	Ort	H_2S	Glu	Man	Xyl	ONPG	Ind	Ure	VP	Cit	TDA	Identified isolate
1	+	+	-	+	+	+	+	+	-	-	-	-	*Escherichia coli*
2	-	-	-	+	+	+	+	+	-	+	-	-	*P. agglomerans*
3	-	-	-	-	-	+	-	+	-	-	-	-	*Klebsiella pneumoniae*

Prevalence of Enterobacteria Isolates

The prevalence of Enterobacteria isolates is presented in Table 3. Microgen identification results shows that various Enterobacteriaceae isolates confirmed were *Pantoae agglomerans* 250 (53.53%) being the most occurring followed by *Klebsiella spp* 160 (34.26%) and then *Escherichia coli* 57 (12.21%) respectively.

Table 3: Prevalence of Enterobacteria Isolates

S/N	Identified isolates	Frequency	Occurrence (%)
1	*Escherichia coli*	74	14.98
2	*Klebsiella* Species	169	34.21
3	*Pantoe agglomerans*	251	50.81
	Total	**494**	**100**

Determination of Multi Drug Resistant (MDR) Isolates

The determination of Multi Drug Resistant (MDR) isolates is presented in Table 4. The result showed that 494 enterobacteria isolates identified were subjected to multi drug resistance (MDR) test, of which 150 (30.4%) isolates were tested to be resistant to the test antibiotics while 344 (69.6%) of the isolates were sensitive.

Table 4: Multi-drug resistant isolates tested

Resistance	No. identified	Percentage Occurrence (%)
Multi-drug resistant	150	30.4
Sensitive isolates	344	69.6
Total	**494**	**100**

Antibiotic Susceptibility Testing

The antibiotic resistance pattern of the Enterobacteriaceae was determined using the most commonly prescribed antibiotics, including Ampicillin (AMP), Amoxicillin (AMC), Ceftazime (CAZ), Chloramphenicol (C), Ciprofloxacin (CIP), Ceftriaxone (CRO), Sulfamethoxazole Trimethoprim (SXT) and Gentamycin (CN). The highest resistance level was recorded for Ampicillin (78%), followed by Amoxicillin (72%), Ceftazidime (42%). There is also a significant level of resistance to Sulfamethoxazole trimethoprim (36%), Ceftriaxone (28%) and chloramphenicol (24%). Lower resistance levels were observed against Gentamicin (8%) and Ciprofloxacin (10%).

Table 5: Susceptibility pattern of the Enterobacteriaceae isolates against some antibiotics

S/N	Antibiotics	Resistance (%)	Sensitive (%)
1	Amoxicillin	108 (72)	42 (28)
2	Ampicillin	117 (78)	33 (22)
3	Chloramphenicol	36 (24)	114 (76)
4	Ceftazidime	63 (42)	87 (58)
5	Ciprofloxacin	15 (10)	135 (90)
6	Gentamicin	12 (8)	138 (92)
7	Ceftriaxone	42 (28)	108 (72)
8	Sulfamethoxazole trimethoprim	54 (36)	96 (64)

DISCUSSION

Respiratory tract infections (RTI) are amongst the most common infections encountered in clinical practice and are a common clinical condition worldwide. But the pattern of antimicrobial resistance varies in different regions. The most common respiratory pathogens identified in this study were *Pantoea agglomerans* (53.5%), *Klebsiella* sp. (34.3%) and *E. coli* (12.2%) respectively. Out of the 1096 total isolates collected from all the study sites, 58 (5.3%) isolates collected were having no growth on subculture while 1038 (94.7%) shows growth on culture media with various cultural characteristics. Some of the test isolates were not identified by the microgen identification GN A ID system software. This might be due to some unknown reasons regarding the kit or due to contamination or the organism being a Gram negative but cannot to be identified by Microgen GN A because it's not been added into the system software. The result of the present study is in line with the work of Yusha'u *et al*., [16], where 20.28% of the Enterobacteriaceae isolates collected from the study site were Gram Positive bacteria after re-identification and Gram staining, while 79.72% were confirmed to be Gram negative bacteria. The report by Mousse *et al*., [17] is similar to the present study where they reported that all the specimens randomly carried to the reference centre during the period were taken as the sample size for the study and were collected following. The present study reveals that *Pantoae agglomerans* (53.5%) was observed to be the most occurring among the isolates within the study sites followed by *Klebsiella* spp (34.3%) with *E. coli* (12.2%) being the least occurring isolate. The result reported by Mousse *et al.,* [17] is not in line with the present study even though some of the isolates observed are common; this might be due to the fact that the bacterial isolates were from different source. From the 1823 collected specimens, 191 strains (10.47%) of Gram negative bacteria were identified. Fifteen different species of Gram negative bacteria were isolated. The species mostly represented were *K. pneumonia* (28.27%), *Acinetobacter* spp. (18.32%), *P. aeruginosa* (15.72%), *E. coli* (14.15%) and *E. cloacae* (12.04%).

The antibiotic resistance pattern of the Enterobacteriaceae was determined using the most commonly prescribed antibiotics, including Ampicillin (AMP), Amoxicillin (AMC), Ceftazidime (CAZ), Chloramphenicol (C), Ciprofloxacin (CIP), Ceftriaxone (CRO), Sulfamethoxazole Trimethoprim (SXT) and Gentamicin (CN). The highest resistance level was recorded for Ampicillin (78%), followed by Amoxicillin (72%), Ceftazidime (42%). There is also a significant level of resistance to Sulfamethoxazole trimethoprim (36%), Ceftriaxone (28%) and chloramphenicol (24%). Lower resistance levels were observed against Gentamicin (8%) and Ciprofloxacin (10%). The result of the present study is contrary to the work of Teklu *et al.,* [18], that reported the highest level of resistance in sulfamethoxazole-trimethoprim (77%), followed by amoxicillin with clavulanic acid (71.6%), cefotaxime (62.2%), ceftazidime (60.%), cefepime (6.3%), norfloxacin (58.8 %), ciprofloxacin (46.3%) and Gentamicin (43.4%). Even though there is significant level of resistance, there is variation in the most resistant antibiotics, which may be because of variation in the sample source, the bacteria identified and the study site as well. The result of this study on the other hand is in line with the findings of studies conducted in Kano, which reported the best activity found in gentamicin with 100% activity against all the test isolates. This is followed by ciprofloxacin (75%). Lowest activity was recorded in cephalothin with 77.5% resistance. Ampicillin was found to be 57.5% resistance while sulfamethoxazole-trimethoprim was 50% resistance. Greater resistance was observed in response to the third generation Cephalosporin i.e. ceftazidime and ceftriaxone having 72.5 % and 40% resistance respectively [19].

CONCLUSION

The study was aimed to determine the antibiotic susceptibility profile of some enterobacteria from respiratory tract infection patients attending some tertiary Hospital in Kano, Northern Nigeria. Based on the result, the highest resistance level was recorded for Ampicillin (78%), followed by Amoxicillin (72%), Ceftazidime (42%). There is also a significant level of resistance to Sulfamethoxazole trimethoprim (36%), Ceftriaxone (28%) and chloramphenicol (24%). Lower resistance levels were observed against Gentamicin (8%) and Ciprofloxacin (10%). It is recommended that, regular surveillance and monitoring is necessary to provide physician's knowledge on the updated and most effective empirical treatment of RTIs.

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