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A REVIEW OF ANTIBIOTIC RESISTANT IN SOME SPECIFIC BACTERIA

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ABSTRACT

The relationship between virulence factors and antibiotic resistance in strains of Y. enterocolitica, biovar 1A is investigated in this work. Serovar O:6, 30-30, and 31 strains were reported to exhibit comparable clavulanic acid resistance. Tetracycline and amoxicillin are the next most common antibiotics that show resistance in Helicobacter pylori infections, after clarithromycin. Antibiotic overuse and self-prescription drive resistance, underscoring the need for more studies and public education initiatives. Additionally, the study discovered that the oral microflora's selection of resistant strains can be accelerated and ecological disruption can result from single-dose antibiotic prophylaxis. The frequency of AMC-resistant Enterobacteriaceae is higher in patients with cystic fibrosis and their siblings. Antimicrobial resistance can result from the coexistence of heavy metals and antibiotics.

KEYWORDS: resistance, antibiotic, strains, antibiotics, result, from, abstract, relationship, between, virulence.

INTRODUCTION

Prophylactic antibiotics are being debated in various medical specialties, including dentistry, to limit antibiotic exposure and prevent antibiotic resistance.[1] One controversial reason for using antibiotic prophylaxis during oral surgery is to avoid infective endocarditis. Recent changes to endocarditis prophylaxis recommendations in the USA and Europe have been questioned, with some researchers suggesting a decrease in antibiotic prescription or an increase in infective endocarditis.[5-8]

The microbiological effects of antibiotics are still unknown, but a study was conducted to determine the ecological makeup and resistance-selection dynamics of normal oral microflora following a single-dose antibiotic prophylaxis, namely 2 g amoxicillin. There are about 50 serovars and 6 biovars of the significant intestinal pathogen Yersinia enterocolitica, which are designated as 1A, 1B, 2, 3, 4, and 5. Research has indicated that strains assigned to biovar 1A encompass many subspecies, leading to their classification as "non-pathogenic."

Amoxicillin-clavulanate (AMC) is the most frequently recommended antibiotic to treat intestinal infections, designed to reverse enteric bacteria's β-lactamase-mediated resistance and restore the effectiveness of β-lactam antibiotics. A recent study found that pathogenicity and resistance to AMC were inversely correlated in clinical isolates of Escherichia coli.

Gram-negative Helicobacter pylori, also known as H. pylori, is a microaerophilic bacteria with four to six polar flagella. Over 50% of people worldwide are infected with H. pylori, with young people in poorer nations more likely to have it. Factors contributing to its rise include poor health, inadequate water supplies, and overcrowding.[25,26]

Antimicrobial agents other than antibiotics can induce antibiotic resistance through a co-selection process.[27,28] Heavy metal pollution is another factor that can cause antibiotic resistance. In certain environments, such as the gastrointestinal system, animal dung, and chicken farming locations, antibiotics and heavy metals coexist, but evidence supporting the combined effects of AMR is not entirely clear.

In this study, the minimum inhibitory concentration (MIC) of amoxicillin was significantly raised by the development of S. aureus in the presence of low concentrations of chromium or cadmium, potentially affecting the emergence of de novo amoxicillin-resistant S. aureus. The study suggests that the development of S. aureus in the presence of low concentrations of chromium or cadmium and amoxicillin may have

contributed to the emergence of de novo amoxicillin-resistant S. aureus from scratch.

A) FECAL ENTEROBACTERIACEAE

Patients with cystic fibrosis (CF) are being treated often with high dosages of various antimicrobial medicines to address chronic endobronchial infections. Recurrent antibiotic treatments have been extensively researched for their impact on the microbiota of the CF lung[1, 2], but less is known about how they affect the microbiome of the digestive tract in this patient population.[3-5] However, it has been demonstrated in studies of populations free of long-term clinical illnesses that antibiotics may cause a significant decrease in metabolically significant bacterial groups[6,7], while other groups, like the Enterobacteriaceae, frequently multiply in response to antimicrobial therapy.[8, 9] Additionally, patients with cystic fibrosis may be more susceptible to resistance gene exchange as a result of the selective pressure of extended antibiotic treatment, which could lead to the spread of antibiotic-resistant strains.[10] In addition, antibiotic medication has the potential to reduce colonization resistance by stimulating the development of preexisting yeasts and/or opportunistic pathogenic bacteria, including Clostridium difficile.[11] These microbes may enter the circulation and result in systemic illness, depending on how severe the dysbiosis is. There is evidence that even brief courses of antibiotic therapy can result in the long-term survival of resistant bacteria in the gastrointestinal tract.[12]

The first-choice antibiotic for treating common infections and moderate respiratory illnesses in individuals who are not hospitalized is amoxicillin (AMX), a broad-spectrum β-lactam penicillin, according to European recommendations.[13] Notwithstanding these recommendations, there has been a notable surge in the past ten years in the use of AMX together with clavulanic acid, a β-lactamase inhibitor, to treat lower respiratory tract infections. For younger individuals with cystic fibrosis (CF), amoxicillin-clavulanic acid (AMC) is used to treat more severe lower respiratory tract infections, such as pulmonary exacerbations caused by Staphylococcus aureus and/or Haemophilus influenzae.[14] The concern about the emergence, development, and dissemination of antibiotic resistance genes grows with the frequent use of these antibiotics. Resistance isolates from the Enterobacteriaceae family, which is commonly linked to abdominal infections, have been repeatedly identified from the intestinal microbiota after the administration of AMX[15, 16] and AMC.[17] As a result, these isolates might present a substantial therapeutic challenge. Therefore, from a therapeutic perspective, it is crucial to learn more about how repeated antibiotic treatments affect the emergence of antimicrobial resistance bacteria in CF patients' guts. The incidence and type of fecal Enterobacteriaceae resistant to ammoniamycin (AMC) were examined in this study in two CF patients and their corresponding healthy siblings.

Two CF patients and their matched healthy sibling provided stool samples for collection (Table 1). A thorough explanation of the supplies and procedures is available as supplemental material. The number of colony forming units per gram fecal sample (CFU/g) from eosin methylene blue (EMB) agar plates containing 0, 8 and 128 ppm AMX was used to determine the prevalence of AMX-resistant Enterobacteriaceae. The two CF patients' samples consistently produced larger counts (6.77 ± 1.06 mean log10 CFU/g) on EMB agar without AMX than the healthy siblings' samples (5.80 ± 0.81 mean log10 CFU/g). The CFU of fecal samples from CF patients did not reduce on plates with 8 ppm AMX (6.91 ± 0.03 mean log10 CFU/g), however the counts of fecal samples from healthy siblings significantly decreased (2.06 ± 0.84 mean log10 CFU/g). The EMB agar counts for CF patient samples on plates containing 128 ppm AMX (6.91 ± 0.03 mean log10 CFU/g) remained within the same range as counts on plates containing 0 or 8 ppm AMX. Counts on plates with 128 ppm AMX dropped to an average of 1.58 ± 3.36 mean log10 CFU/g for samples of healthy siblings.

TABLE 1

Overview of clinical characterstics of volunteers, isolates, MALDI-TOF MS result, sequencing results and MICs.

Volunteer	Date of birth	Sampling date	Sample code	Antibiotic history a		No. of isolates	MALDI-TOF MS clusters	Final identification	Source (µg/ml AMX) b	MIC range	
				Antibiotic agent, route and doses	Administration period					AMXc	AMCc
Patient 1	12/03/2005	17/02/2008	P1-s1	Augmentin PO 3 x 4 ml	15/10/2007 – 01/05/2008	80 18	IV VII	Klebsiella oxytoca E. coli	4 128 256 4 128	- 256 >256 >256 >256	2/1 - 2/1 32/16 32/16
		04/08/2008	P1-s2	Augmentin PO 3 x 225 mg	10/07/2008-01/08/2008	21 13 10 13	IV V VI VII	Klebsiella oxytoca Klebsiella variicola Citrobacter spp. E. coli	128 128 16 128 16 128	>256 >256 >256 >256 >256 >256	163/8-32/16 8/4-16/8 32/16 32/16 8/4 4/2-32/16
		2/05/2009	P1-s3	Augmentin PO 3 x 5 ml Duracef PO	03/002/2009-10/02/2009 10/0200/2009-23/04/2009	0[d]	-	-	-	-	-

				3 x 5 ml Pobramycin INH 2x1 amp Ciproxin PO 3 x 150 mg Colistine b INH 2x2 million units	13/02/2009-12/03/2009 23/04/2009-23/07/2009 23/04/2009-23/07/2009						
Sibling-1	20/09/2001	17/02/2008 04/08/2008 02/05/2009	S1-s1 S1-s2 S1-s3	No >1.5 years No No	N/A N/A N/A	20 4 15	III III I	E.coli E.coli E.coli	1 8 -	4 8 -	2/1 4/2 -
Patient 2	02/07/1198	01/12/2007	P2-s1	Augmintine PO 4 x 250 mg	27/11/2007-04/12/2007	3 15	I II	E.coli	256 256 128	>256 >256 >256	8/4 8/4 -
Sibling 2	07/05/1992	02/12/2007	S2-s1	No> 3 years	N/A	2	I	E.coli	1	8	2/1

A total of 173 patient isolates and 41 sibling isolates were collected after enumeration. The sample P1s3 yielded no isolates. In spite of the medium containing an antifungal drug, this sample yielded solely yeast colonies. Time of Flight for Matrix-Assisted Laser Desorption Ionization Based on their mass spectra, mass spectrometry (MALDI-TOF MS) was utilized to dereplicate the purified isolates. After visually assessing the location and intensity of the mass spectra's peaks, seven clusters were identified at a 75% Pearson similarity (Fig. S1). Representatives from each cluster were then chosen for additional taxonomic analysis.

All of the chosen isolates were shown to belong to the Enterobacteriaceae family by partial 16S rRNA gene sequence analysis (data not shown). Partial rpoB sequence analysis was then employed to identify the organism at the species level. Isolates of MALDI-TOF MS clusters I, II, III, and VII were identified as Escherichia coli, and members of cluster IV were identified as Klebsiella oxytoca based on the results of the BLAST analysis (N99% rpoB sequence similarity with the type strain), the topology of the rpoB sequence-based ML tree (Fig. S2), and API-20E tests (bioMérieux) (data not shown). Cluster V strains were rpoB sequence-assigned to the closely related species group Klebsiella variicola/pneumoniae/singaporensis (N98% sequence similarity towards the type strains). However, based on the topology of the rpoB sequence-based ML tree, the strains are most closely positioned to K. variicola (Fig. S2). Also, the BLAST analysis was unable to identify a single representative isolate of cluster VI.

This strain was placed in the genus Citrobacter based on the topology of the rpoB sequence-based ML tree and the API-20E findings (BioMerieux). While the 173 patient isolates were more diverse in terms of taxonomy, including members of K. oxytoca (58.4%), E. coli (28.3%), K. variicola (7.5%), and Citrobacter sp. (5.8%), all 41 sibling isolates were classified as E. coli. Within the relatively tiny Enterobacteriaceae population, E. coli is a major member in healthy gut populations. But following antibiotic therapy, a shift in species evenness is frequently noticed, which is frequently marked by a decrease in E. Coli counts and an increase in the prevalence of Klebsiella sp., Enterobacter sp., and/or Citrobacter sp.[18,19] AMX MICs were found for a selection of 24 CF isolates and 5 isolates from sibling samples, which were chosen based on the isolation source and MALDI-TOF MS clustering. Furthermore, AMC MICs for three sibling isolates and twenty CF isolates were found. Based on the CLSI breakpoints, all five of the sibling isolates that were chosen for the AMX MIC determination had MICs that were lower than or equal to 8 µg/ml, and the three that were chosen for the AMC MIC determination had MICs that were lower than 8/4 µg/ml. On the other hand, AMX MICs of N256 µg/ml were found in all 24 tested CF-isolates, suggesting a significant degree of resistance to AMX.

Nine isolates of CF patients with MIC values of 32/16 µg/ml were resistant in AMC testing. (Fig. 1). AMC MICs of 16/8 µg/ml were found in 4 CF isolates, showing intermediate resistance, while AMC susceptibility was seen in the remaining 7 isolates. K. oxytoca was found to be the predominant strain in cluster IV, which contained 58.4% of all CF isolates. After taking AMX or AMC, overgrowth of the intestinal microbiota with Klebsiella species is frequently observed.[15,16] All six of the K. oxytoca isolates that were chosen for this study had high levels of resistance to AMX (MIC N 256 µg/ml). This species has been previously demonstrated to be able to manufacture β-lactamases that are chromosomally encoded, ideally rendering penicillins like AMX inactive.[20] Furthermore, mutations in the blaOXY-1 and blaOXY-2 promotors of the β-lactamase gene might result in an excess of β-lactamase enzymes, which can lead to resistance to extended-spectrum β-lactam antibiotics. K. oxytoca was isolated in P1 at two distinct sample locations. All four of the selected isolates from the second sample site were intermediately to highly resistant to AMC, while the two isolates from the first sampling point were responsive to the drug. Although it should be emphasized that this

patient also got the same antibiotic therapy before to the first sampling, it is possible that this resistance was brought on by the antimicrobial therapy administered between the two sampling sites (Table 1). Following antibiotic therapy with AMX, MICs among fecal Klebsiella species have been shown to increase by Adamsson and colleagues.[21]

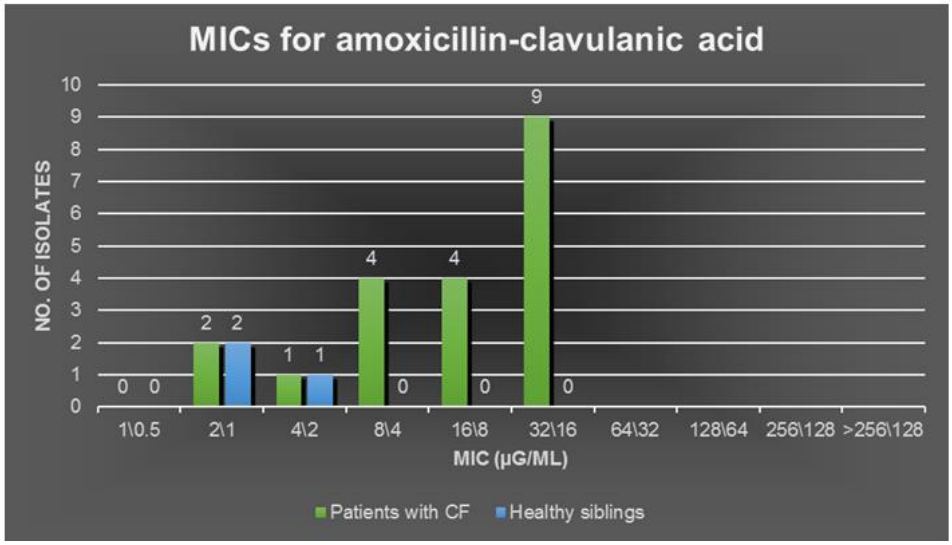

Fig. 1: Distribution of amoxicillin–clavulanic acid MIC values (µg/ml) for isolates obtained from 3 patients (n = 20) with CF and 3 healthy siblings (n = 3). Brackets indicate interpretive breakpoints following CLSI criteria: susceptible] intermediate resistance [resistant.

Furthermore, K. variicola accounted for 7.5% of all isolates for CF. A high degree of AMX resistance was seen in three typical isolates. Furthermore, two of these isolates displayed an intermediate degree of AMC resistance. Of the isolates with CF, 28.3% contained E. coli. Five of the seven chosen E. Col. isolates from P1 were resistant to AMC, but three of the chosen isolates from P2 were susceptible to it. Additionally, the fact that these AMC-resistant isolates were found in samples from two distinct P1 sampling points may suggest a prolonged persistence. The primary mechanism of AMX/AMC resistance in E. coli strains is the hyper-production of TEM1 β-lactamase, which is encoded on plasmids.[22] Cluster VI isolates were categorized as Citrobacter and accounted for 5.8% of all CF isolates. This cluster's isolates were all from P1-s2, and they all showed significant resistance to both AMX and AMC. This may be explained by the innate resistance of Citrobacter species to AMX, AMC and narrow-spectrum cephalosporins through chromosomally encoded AmpC β-lactamase enzymes.[23] As compared to their healthy siblings, the fecal microbiota of two CF patients showed a significantly greater prevalence of AMX and AMC resistant Enterobacteriaceae, according to the preliminary study's findings.

This lends credence to the theory that frequent administration of AMC (Table 1) may be a significant contributing factor to the elevated levels of antibiotic-resistant subpopulations in the gastrointestinal microbiota of these individuals. Thus, a higher concentration of resistant Enterobacteriaceae in their feces could pose an additional infectious risk to these patients and serve as a significant reservoir for the dissemination of resistance determinants. This has to be confirmed by expanding the small range of our dataset to include additional patients and antimicrobial drugs, as well as by concentrating on other intestinal microbiota members.

B) ORAL MICROFLORA AND SELECTION OF RESISTANCE AFTER A SINGLE DOSE OF AMOXICILLIN

METHOD

Study population

Thirty-three healthy participants between the ages of 18 and 45 were enrolled in the study (17 male and 16 female). An notice at the Department of Dental Medicine, Karolinska Institutet, Stockholm, Sweden, was used to enlist volunteers. Due to insufficient sample collection, four volunteers were eliminated, leaving 29 participants with a mean age of 30 years in the research population.

Study design

Before drinking, brushing their teeth, or smoking in the morning, participants in the fast were asked to provide a 5-mL unstimulated saliva sample (day 1, control sample). One pill containing two grams of amoxicillin was given orally as a single dosage while being closely monitored. Samples from days 2, 5, 10, 17, and 24 were then obtained independently by participants following thorough written and verbal instructions.

Statistical analysis

Sample size (n ¼ 30) plus an extra 10% to account for potential dropout (n ¼ 3) was calculated via a power calculation.[24]

The Wilcoxon signed rank test was used to compare changes in the median log number of bacteria per milliliter of saliva to the baseline value (day 1, control sample). A paired two-tailed t-test was used to compare changes in the percentage of bacteria that were less susceptible to amoxicillin. P values less than 0.05 were considered significant.

RESULT

Ecological disturbance on oral microflora

The spread of oral microflora over the course of the investigation is displayed in Table 1. The amount of viruses that cause streptococci, including Streptococcus salivarius on days 2 and 5 (p <0.001), Streptococcus sanguis and Streptococcus anginosus on day 2 (p 0.04), was significantly reduced. Day 2 saw a significant increase in Neisseria spp. (p 0.02). No other aerobic or anaerobic species showed any appreciable alterations.

TABLE 1

Distribution of aerobic and anaerobic oral microflora throughout the study period.

	n[a]	Day 1 Median (min-max)	n[b]	Day 2 Median (min-max)	n[b]	Day 5 Median (min-max)	n[b]	Day 10 Median (min-max)	n[b]	Day 17 Median (min-max)	n[b]	Day 24 Median (min-max)	n[b]
Aerobic bacteria													
Streptococcus salivarius spp.	29	16.8 (10.1-19.1)	29	12.6 (0-18.1)	23	13.1 (6.9-18.8)	29	16.11 (7.82-19.11)	29	15.76 (9.62-18.83)	29	16.1 (5-20)	29
Other viridants streptococci	29	17.7 (13.1-19.7)	28	16.5 (12.4-19.1)	29	16.8 (10.8-19.1)	28	17.73 (13.12-19.52)	29	17.73 (12.43-20.03)	29	17.9 (14.5-22.5)	28
Neisseria spp.	25	15.8 (9.2-17.4)	18	15 (10.8-20)	25	15.4 (11.5-17.7)	19	16.12 (11.51-18.83)	21	14.22 (7.31-17.73)	22	15.4 (9.2-18.4)	21
Micrococcus spp.	9	16.8 (14.4-20.0)	9	15.5 (13.1-16.5)	7	13.8 (11.5-17.7)	8	17.03 (11.92-18.42)	9	14.73 (11.92-17.37)	7	16.1 (14.2-17.7)	9
Staphylococcus aureus	8	10.8 (7.3-18.1)	7	11.5 (8.5-15.4)	6	13.7 (6.9-18.4)	6	10.46 (9.21-14.29)	7	12.32 (8.52-15.42)	8	10.8 (7.3-15.4)	5
Candida spp.	3	7.8 (6.9-8.8)	3	7.6 (7.3-8.4)	3	7.3 (6.2-8)	3	6.76 (6.21-7.31)	2	7.46 (7.31-7.60)	2	8.2 (8-8.3)	2
Enterobacteriacae spp.	3	9.4 (8.3-10.8)	3	7.3 (7.3-7.3)	2	8.1 (7.6-8.5)	2	10.82	1	17.73	1	9 (7.6-15.4)	3
Staphylococcus epidermidis	2	10.1	1		0	15.5	1	16.81	1	12.8 (8.52-17.03)	2	17 (16.5-17.4)	2
Haemophilus spp.	1		1		0		0	15.42	1		0		0
Corynbacterium spp.	1		0	14.2	1	14.7	1		0		0		0
Enterococcus spp.	1		0		0		0		0	9.2	1	10.8	1
Anaerobic bacteria													
Prevotella spp.	27	15.4 (9.6-19.7)	26	13.8 (8.3-19.1)	27	14.5 (6.9-19.3	20	13.5(8.5-19.1)	25	13.8 (6.9-18.4)	24	13.5 (6.2-19.3)	27
Leptotrichia spp.	19	8.5 (6.2-17.7)	15	9.21 (6.2-13.1)	16	9.2 (7.3-13.1)	17	9.2 (6.2-12.4)	16	8.4 (6.2-17)	17	8.7 (6.2-15.4)	19
Lactobacillus spp.	13	8.3 (5.30-10.82)	13	9 (7.3-17.7)	13	8.5 (4.6-10.6)	12	8.9 (6.2-9.2)	12	7.6 (6.2-10.1)	13	8.5 (6.2-10.8)	12
Fusobacterium spp.	13	9.2 (6.2-12.6)	8	8.3 (6.2-12.4)	13	9.3 (6.2-15.4)	9	17.5 (15.4-18.4)	7	8.9 (6.2-14.4)	7	12.5 (8.5-15.4)	10
Veillonella spp.	8	17 (9.2-19.1)	8	14.7 (13.1-17.7)	4	14 (6.9-17.5)	7	13.5 (10.8-16.1)	7	15.4 (8.5-20)	6	10.3 (9.3-12.9)	5
Anaerobic cocci spp.	5	17.2 (7.3-17.4)	5	13 (12.6-13.4)	2	13 (10.1-13.8)	3	10.8 (9.7-11.3)	4	11.3 (6.2-11.7)	3	4.7 (3.5-15.8)	4
Actinomyces spp.	3		0	14.5	1	16.5	1	7.6	1	15.9	1		0

Median is the median of log values of the number of microorganisms above the detection level per mL saliva.
a Number of patients with detectable levels of microorganisms within the sampling period.

b Number of patients with detectable levels of microorganisms at the actual sampling day.

Antibiotic-resistant microorganisms

Amoxicillin-resistant viridans streptococci were isolated prior to antibiotic administration in 21% (n = 6/29) of the participants. Prior to the administration of antibiotics, 59% (n ¼ 17/29) of amoxicillin-resistant Prevotella spp. were carriers.

On days 2 and 5 (p 0.004 and p 0.04, respectively), there was a substantial rise in the fraction of viridans streptococci with decreased susceptibility to amoxicillin.

It was found that during the study period, approximately one-third of the individuals developed resistant viruses against amoxicillin, clindamycin, and penicillin-V (Table 3). Across all studied antibiotics, Prevotella spp. showed a 28% increase in resistance.

CONCLUSION

In summary, the natural oral microbiota experienced an ecological disruption and a notable selection of resistant strains was brought about by a single dose of amoxicillin. Moreover, the prevalence of amoxicillin-resistant streptococci in the oral cavity of healthy people may be underestimated. The significance of assessing the effectiveness of antibiotic prophylaxis in various surgical specialties and balancing any potential advantages against the potential for microbiological repercussions is underscored by this instance.

C) YERSINIA ENTEROCOLITICA BIOVAR IA

METHODS

1) The minimum inhibitory concentrations (MICs) for AMC were compared with the presence or absence of VFs in six clinical strains of Y. enterocolitica biovar 1A of two serotypes, namely O: 6, 30 and O: 6, 30-6; 31.
2) Table 1 provides an overview of the strains' characteristics, including their VFs, MICs for AMC, and laboratory accession numbers.

TABLE 1: Details of AMC-susceptibility and presence of virulence- related factors in strains of *Yersinia Enterocolitica* Biovar 1A.

			Virulence-related factors				
Strains	**Serotypes**	**MIC for AMC (mg/L)**	**has A**	**Type 1 secretions protein**	**Flagellar hook protein**	**ystB**	**tccC**
C16	O:6,30-6,31	>256	-	+	-	+	-
C17	O:6,30-6,31	>256	-	-	-	+	-
C27	O:6,30-6,31	192	-	-	-	+	-
C927	O:6,30	192	+	+	+	+	-
C764	0:6,30	192	+	-	+	+	-
C855	0:6,30	62	-	-	+	+	-

hasA: Hemophore A, ystB: Yersinia stable toxin B, tccC: Insecticidal toxin, AMC: Amoxicillin-clavulanate, MIC: Minimum inhibitory concentration.

RESULTS AND DISCUSSION

1) We found that the serovar O: 6, 30–6–31 strains displayed a comparable association between the number of VFs and clavulanic acid resistance, as noted by Oteo et al. for E. coli clinical isolates.

2) Serovar O strains had less virulence-related genes (6, 30–6, 31), but they were more resistant to the β-lactamase inhibitor, which raised the AMC's minimum inhibitory concentration.

3) Nevertheless, isolates of serovar O: 6, 30 showed a variable correlation between VF count and clavulanic acid resistance.

4) For every strain reported here, we also examined the promoters and complete coding sequence (CCDS) of the β-lactamase gene blaA in a prior work.

5) Based on this, we deduced that the blaA of serovar O: 6, 30-6, 31 and the promoters and CCDS of the β-lactamase gene (blaA) of all three strains of serovar O: 6, 30 were the same.

6) It has also been proposed that host adaptation and genetic alterations led to the formation of clinical strains of bioserovar 1A/O: 6, 30–6, 31 from environmental strains.

Hence, features other than serotypes or differences in the gene sequences of β-lactamases may influence the association between resistance to AMC and virulence-related parameters.

7) This is the first study to look at the connection between VFs and AMC resistance in Y. enterocolitica biovar 1A strains.

D) HELICOBACTER PYLORI BACTERIA OBTAINED DISEASE

METHOD

This descriptive-analytical study assessed the drugs' resistance to Helicobacter pylori in biopsy samples from 205 individuals. The study evaluated the relationship between antibiotic resistance and risk variables such age, sex, job status, treatment history, and reason for referral. It employed a census sample approach. At least 95% confidence was placed on the sample size estimate of 205 patients.

Four age categories were used to categorize the research population: under 30, 30–40, 41–50, and above 50. Every patient had three biopsy samples obtained, and one sample was used for the quick urease test. For the transfer, sterile tubes holding 500 µl of physiological saline were utilized. Samples that tested positive for urease were crushed in a saline solution and fed to Brucella agar medium that had 10% sheep blood and a particular supplement that included B amphoteric antibiotics, trimethoprim, and vancomycin.

Cellular features in hot staining, colony morphology, bacteriological morphology, urease activity, catalase, and oxidase activity were used to identify and diagnose Helicobacter pylori. Both descriptive statistics and logistic regression were employed to examine the association between antibiotic resistance and independent factors. Software for data analysis included R and SPSS. The Lorestan University Research Ethics Board accepted the project, which followed STROCC 2021 rules.

RESULT

205 out of 608 samples examined for Helicobacter pylori infection were positive. The patients' mean age was 42.32 ± 16.65 years, and 29.8% of them were under 30. With 99.5% of the patients being city dwellers and 33.2% being self-employed, the bulk of the patients were men. Epigastric discomfort was the most frequent cause for referral, which was followed by dysphagia, heartburn, nausea, vomiting, appetite loss, weight loss, acid reflux disease, and blood in the stools. The research revealed a low treatment history, with 9.8% reporting a history of therapy and 7.3% reporting a history of using antibiotics to treat Helicobacter pylori. The study emphasizes how crucial it is to comprehend the clinical characteristics of individuals infected with Helicobacter pylori.

The frequency distribution of patients' antibiotic sensitivity and resistance was assessed in the study. It was discovered that 53.2% of patients had amoxicillin-sensitive culture findings, whereas 46.8% of patients reported amoxicillin resistance. Of the culture findings, 41% had tetracycline resistance and 59% had tetracycline sensitivity. Patients between the ages of 41 and 50 had the least resistance, with 36.8% having the most resistance.

Tetracycline resistance was lowest in patients aged 41–50 years, at 11.9%, and highest in those under 30 years old, at 34.5%. Tetracycline resistance did not substantially correlate with age groups. The majority of tetracycline-resistant patients reported having epigastric discomfort, and there was no discernible variation in the patients' reasons for referral.

The majority of individuals (32.3% and 11.7%, respectively) with metronidazole resistance were found to be between the ages of 31 and 40. Metronidazole resistance was not substantially correlated with employment status. The majority of patients worked for themselves, and metronidazole resistance was not associated with the reason for referral.

The proportion of patients with clarithromycin resistance was highest (35%), in patients under 30 years old, and lowest (41–50 years old). Antibiotic resistance did not significantly differ between the age categories. Clarithromycin resistance was present in 63.4% of females, which did not differ substantially from male gender. The majority of patients were housewives, and levofloxacin resistance was not a factor in the referral's cause.

Age-wise, bismuth resistance was lowest in patients 41–50 years old (15%) and highest in those under 31–40 years old (35%). Bismuth resistance did not substantially differ across the age categories of the patients, nor did it significantly differ between male and female patients (50% and 50%, respectively).

The study concludes that in order to enhance patient outcomes, it is critical to comprehend the frequency distribution of antibiotic resistance and sensitivity in patients.

35% of patients with bismuth resistance were self-employed, whereas 2.5% of patients who had previously been treated showed the least amount of resistance. Among patients, the difference between those who lived in an urban (97.5%) and rural (2.5%) environment was also not significant, $p = 0.19$. $P = 0.64$ indicates that there was no discernible relationship between bismuth resistance and work status. The majority of patients (47.5%) who had bismuth resistance reported having epigastric discomfort; no patient who had blood in their stools had bismuth resistance.

Regarding the basis for referral, there was no statistically significant difference in bismuth resistance among the patients ($p = 0.58$). Tetracycline resistance did not differ between patients with (5%) and without (95%) a history of therapy, $p = 0.38$.

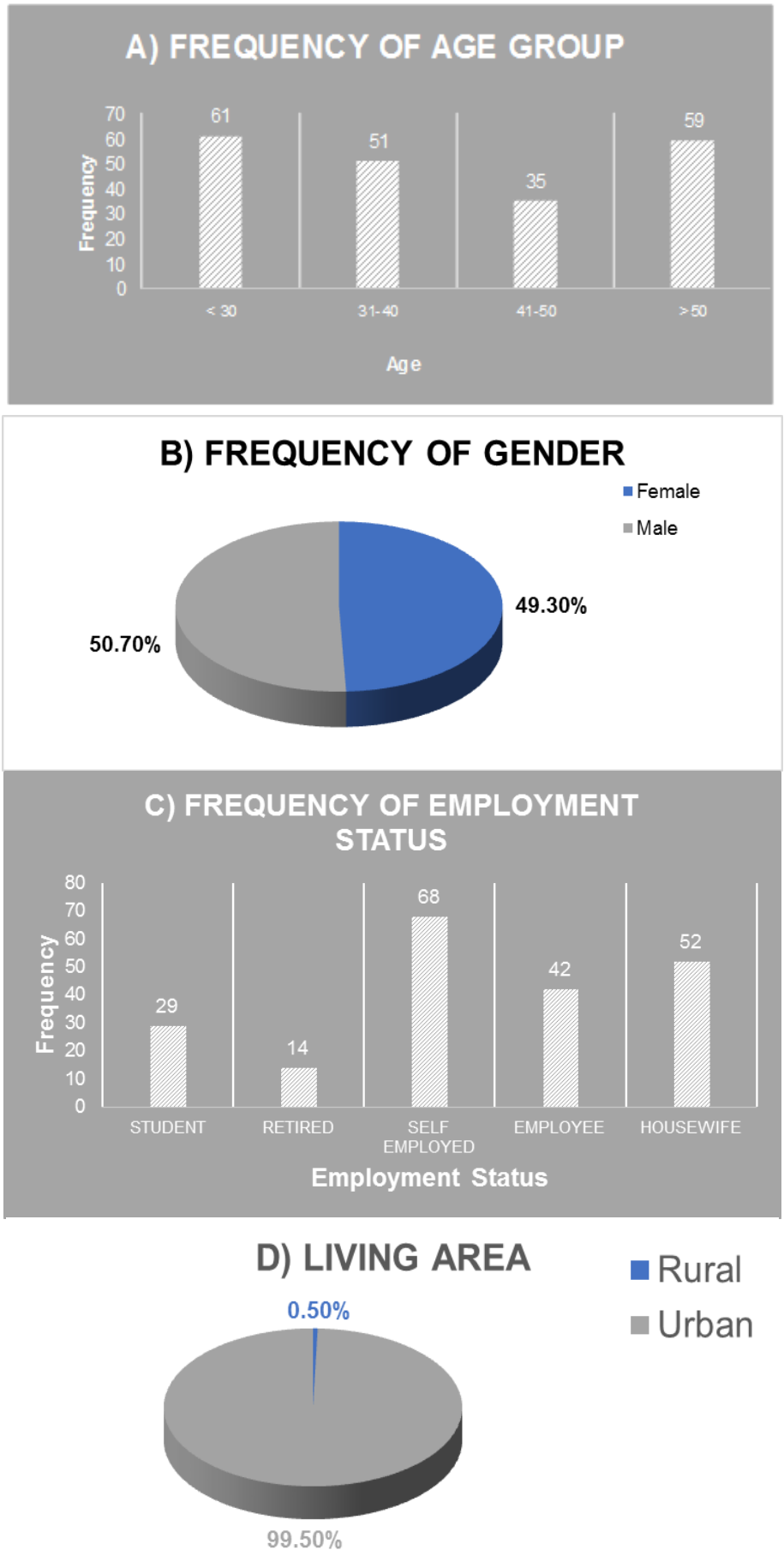

Fig. 1: Frequency distribution of demographic characteristics of the studied patients.

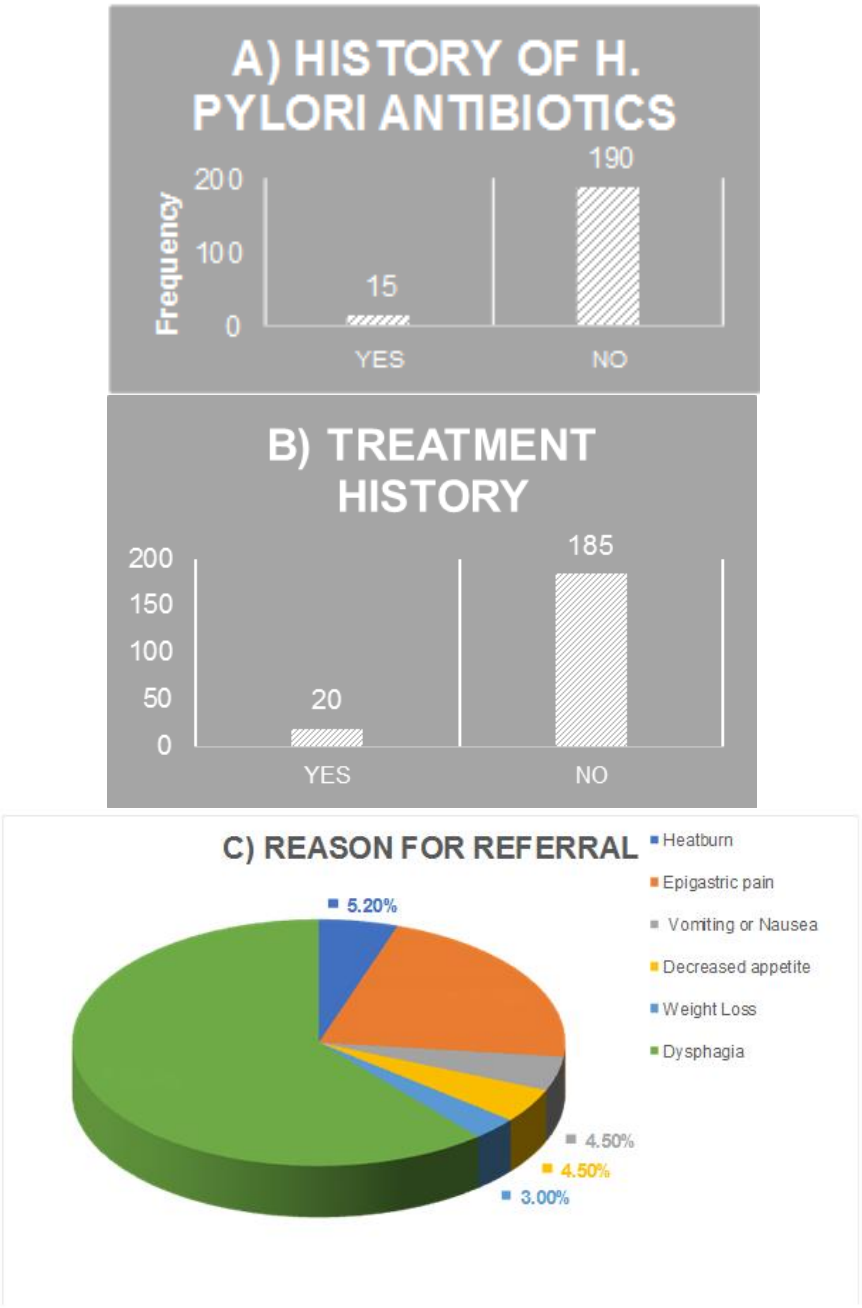

Fig. 2: Frequency Distribution of Clinical Features of Patients Studied.

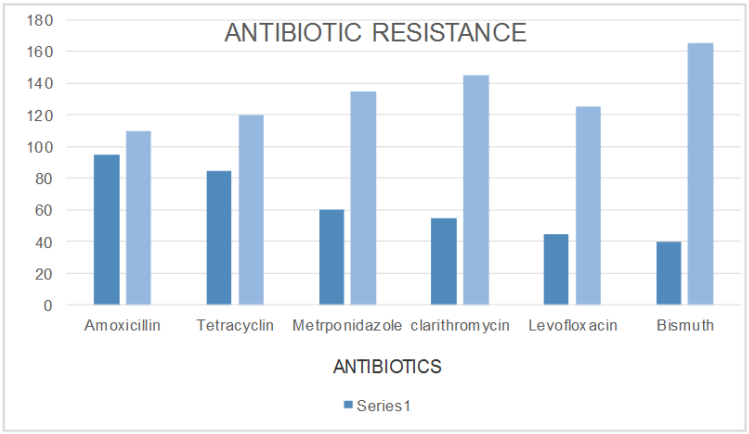

Fig. 3: Frequency of antibiotic resistance and sensitivity in the studied patients.

DISCUSSION

A Helicobacter pylori infection is a major cause of gastrointestinal disorders, and studies conducted in vitro have demonstrated the effectiveness of several antibiotics in inhibiting the growth of this bacteria. Geographically, the prevalence of h. pylori infection ranges from 20 to 40% in Scandinavian nations and the United Kingdom, while it is above 80% in Japan, South America, Turkey, and Pakistan. The average age of the patients involved in this research was 42.32 years.

Metronidazole was the first antibiotic used to treat h. pylori, and it has been noted that this drug can lead to the emergence of extremely resistant strains. The percentage of people resistant to metronidazole was 33.2%, which is less than the norm for Africa (97.5%) and about the same as the average for Europe (34.2%).

Amoxicillin resistance is extremely low; in Iran, the largest and lowest percentages of resistance are found in Tabriz (28.6%) and Tehran (1.6%). The current investigation found that h. pylori resistance to amoxicillin was 46.8%, which was more than the 59% found in a randomized controlled trial by Caliskan et al.

The rate of tetracycline resistance is rising; in Tehran, the highest number was 38.1%. Compared to the findings of comparable research, the population's level of clarithromycin resistance was significantly greater at 70.7%. Treatment for H. pylori infections also involves the use of fluoroquinolones, such as ciprofloxacin, moxifloxacin, trovafloxacin, and levofloxacin.

Helicobacter pylori's sensitivity to antibiotics can be attributed to a variety of causes, including incorrect drug selection, antibiotic misuse, inaccurate treatment duration calculations, laboratory failure to produce the bacterium, and noncompliance with recommended protocols.

CONCLUSION

The study's findings demonstrate that patients' resistance to standard antibiotics used in treatment regimens to treat Helicobacter pylori is growing. Higher than other drugs, h. pylori's resistance to clarithromycin, amoxicillin, and tetracycline is uncorrelated with patient- and demographic-related factors. Prior to therapy, culture and susceptibility tests are required to ascertain the drug resistance patterns of this bacteria in various geographic locations.

E) STAPHYLOCOCCUS AUREUS

METHODS AND MATERIALS

Collection of Staphylococcus aureus

In this work, the Food Microbiology Laboratory, Laboratory Sciences and Services Division, International Center for Diarrheal Disease Research, Bangladesh (ICDDR,B) recovered Staphylococcus aureus from processed raw beef (meat balls) from a nearby restaurant.

Pre-screening of antibiotic susceptibility pattern

Mueller-Hinton agar plates were pre-inoculated with chromium or cadmium-exposed S. aureus, and then the standard antibiotic discs, Azithromycin (15 µg), Chloramphenicol (30 µg), Ampicillin (10 µg), Amoxicillin (10 µg), Erythromycin (15 µg), Doxycycline (30 µg), Ciprofloxacin (5 µg), Cephradine (30 µg), Clindamycin (2 µg), Methicillin (5 µg), and Vancomycin (30 µ) were impregnated on Mueller-Hinton agar plates.

Determination of bacterial growth kinetics in heavy metal and antibiotic co-exposure setting

10 µL (equivalent to 108 cfu/mL) of bacterial suspension was inoculated in 0.5 mM chromium, 0.025 mM cadmium, or with 0.06 µg/mL of amoxicillin (e.g., the minimal inhibitory concentration) containing TSB media. The mixture was then incubated in a shaker incubator at 37°C and 180 rpm to ascertain the co-exposure effect of chromium or cadmium and amoxicillin on S. aureus growth. As previously mentioned, the optical density of was determined at 600 nm.[29]

Determination of minimum inhibitory concentration of amoxicillin for co-exposed S. aureus

S. aureus was cultured in TSB for 48 hours at a 12-hour interval, either with or without 0.5 mM chromium and a low dose of amoxicillin (0.06 µg/mL). Similarly, S. aureus was cultivated in a co-exposure setting with modest concentrations of amoxicillin (0.06 µg/mL) and cadmium (0.025 mM).

Determination of efflux activity of chromium or cadmium pre-exposed reference strain of S. aureus

A low concentration of chromium or cadmium was pre-exposed to S. aureus ATCC #6538 for 72 hours, with 12-hour intervals between subculturings. After that, a bacterial solution containing 100 µL (or 107 cfu) was inoculated for 30 minutes in PBS that was supplemented with 2 µg/mL ethidium bromide (EtBr) and 0.25% glucose. The efflux activity was measured using a semi-automated fluorometric approach on a fluorescence plate reader (Agilent BioTek Synergy LX) at 5-minute intervals for 30 minutes, with 530 nm excitation and 600 nm emission spectrums. This was done after changing the buffer with EtBr-free PBS with or without glucose.

Extraction of RNA from S. aureus *isolates and cDNA synthesis*

Because S. aureus has a modified peptidoglycan layer that makes it resistant to lysozyme[30], the simplest phenol technique is the most efficient way to lyse bacterial cells and retrieve the largest amount of RNA. Bacterial RNA was isolated using the Monarch Total RNA Miniprep Kit (New England Biolab) following phenol treatment. RNA quality was assessed using agarose gel electrophoresis. Next, using the ProtoScript II First Stand cDNA synthesis Kit, cDNA synthesis of extracted RNA was carried out.

Quantification of mRNA level of norA, mepA and femX using reverse transcription polymerase chain reaction (RT-qPCR)

As mentioned in Section 2.6, S. aureus was cultured for 48 hours in a chromium and amoxicillin coexposure environment in order to measure the expression levels of norA, mepA, and femX. Using the aforementioned generated cDNA, reverse transcription polymerase chain reaction (RT-qPCR) was used to calculate the cDNA copy number of each gene (Table 4). The following PCR primers (Tables 1 and 2) and PCR-master mix were used for the RT-qPCR.

TABLE 1

Primer sequence, size and product size.

Name of gene	Primer Sequence	Length (bp)	Product size (bp)
femX	5'GCGAAGAATCGCTGTAGGTC3' 5'TGCATACGCTTTCTCAGCTT3'	20 (forward) 20 (reverse)	193
norA	5'TGGCCACAATTTTCGGTAT3' 5'CACCAATCCCTGGTCCTAAA3'	20 (forward) 20 (reverse)	182
mepA	5'TGCTGCTGCTCTGTTCTTTA3' 5'GCGAAGTTTCCATAATGTGC3'	20 (forward) 20 (reverse)	198
GAPDH	5'TGACACTATGCAAGGTCGTTTCAC3' 5'TCAGAACCGTCTAACTCTTGGTGG3'	24 (forward) 24 (reverse)	180

TABLE 2

List of reagents for RT-qPCR.

Name of reagent	Volume
Template : cDNA	1.0 µL
SYBR-Green master mix	10 µL
1 µM primer Forward (25nM)	1.0 µL
1 µM primer Forward (25nM)	1.0 µL
Nuclease free water	7.0 µL
Total volume	20 µL

Statistical analysis

For all statistical studies, GraphPad Prism version 6.0 was used. The Sidak multiple-comparison test and analysis of variance (one-way ANOVA) were run. The standard error of mean, or mean ± SEM, was used to express the data. $P < 0.05$ values were regarded as statistically significant.

RESULTS

3.1. Isolation of Staphylococcus aureus *and culture Staphylococcus aureus* (*S. aureus* was isolated by the Food Microbiology Laboratory, Laboratory Sciences and Services Division, International Centre for Diarrhoeal Disease Research, Bangladesh, from processed raw meat (meat balls) from a nearby restaurant. The ATCC #6538 standard reference strain of S. aureus was obtained from the Microbiology Department of the University of Alabama in Birmingham, USA. S. aureus is a facultative anaerobic bacteria and a gram-positive coccus. It grows well in tryptone soya medium and is not a fussy eater. Using the Miles-Mishra serial dilution method, the average viable count in this medium at mid-log phase (O.D. 0.5 at 600 nm) was roughly 108 cfu/mL.

Growth kinetics of* S. aureus *in the presence of chromium salt

In TSB enriched with or without chromium (Cr6+) salt, S. aureus was cultivated. The growth curve showed that S. aureus could survive in a medium containing 3 mM Cr6+ salt; however, at 5 mM or greater concentrations, the bacterial growth was hindered. Because this strain of S. aureus can thrive at these concentrations, 0.5 to 3.0 mM chromium salt was utilized in exposure tests. In a similar vein, we discovered that S. aureus was viable in a cadmium (Cd2+) salt solution up to 0.1 mM; but, at ≥0.3 mM, the bacterial growth was suppressed (Fig.1B). Thus, when evaluating S. aureus exposure, a cadmium salt solution with a concentration range of 0.005 to 0.1 mM was employed.

Antibiotic sensitivity pattern of* S. aureus *after exposure to chromium/cadmium

After being exposed to a range of 0.5 to 1.0 mM Cr6+, S. aureus isolates showed a considerable drop in their zone of inhibition (ZOI) for amoxicillin, from 36 mm to 27 mm, according to the antibiotic susceptibility profile. In a similar vein, S. aureus isolates exposed to chromium showed a substantial reduction in the ZOI for ciprofloxacin. Moreover, following 48 hours of exposure to a range of 0.05 to 0.1 mM Cd2+, the ZOI in S. aureus isolates against amoxicillin was drastically reduced from 37 mm to 30 mm. Regarding the other antimicrobials examined, there was no discernible shift in the ZOI (Table 3).

Co-exposure effects of chromium or cadmium and a low concentration of amoxicillin on* S. aureus *growth

Tryptone soya broth was used to cultivate S. aureus, either with or without 0.5 mM chromium salt, 0.06 µg/mL amoxicillin, or both. In terms of quantitative results, the application of either amoxicillin or chromium treatment alone reduced the rate of bacterial growth by 52.3% relative to the control. However, the combination of 0.5 mM chromium salt and 0.06 µg/mL amoxicillin increased the rate of bacterial growth by up to 77.3%. Overall, S. aureus growth kinetics showed that preexposure to chromium reduced the amoxicillin's inhibitory impact, indicating that chromate increased amoxicillin resistance. Additionally, the midlog phase growth kinetics revealed that while co-exposure to both chromium and amoxicillin significantly boosted bacterial growth, bacteria exposed to amoxicillin showed lower

growth relative to isolates with exposure. The curve showed that a single exposure to amoxicillin reduced bacterial growth by 52.3% when compared to S. aureus that had not been exposed, whereas exposure to cadmium in combination with a low dosage of amoxicillin increased growth rate by 82.6%. Overall, S. aureus growth kinetics indicate that, in comparison to a single exposure to either heavy metal or antibiotic, the growth pattern of S. aureus was changed after exposure to both.

TABLE 3
Zone of inhibition of s.aureus against clinically important antibiotics following exposure to chomius or cadmium.

Name of antibiotic	Antibiotics susceptibility of s.aureus with and without exposures to chromium salt			Antibiotics susceptibility of s.aureus with and without exposures to cadmium salt		
	Level of chromium	ZOI (mm)	P value	Level of cadmium	ZOI (mm)	P value
Amoxicillin	0mM 0.5mM 1.0Mm	36 30 27	0.05	00 0.5mM 0.1mM	37 33 30	0.01
ciprofloxacin	0mM 0.5mM 1.0Mm	35 34 30	0.05	00 0.5mM 0.1mM	32 30 28	NS
Azithromycin	0mM 0.5mM 1.0Mm	29 29 26	NS	00 0.5mM 0.1mM	27 26 26	NS
chloramphenicol	0mM 0.5mM 1.0Mm	28 27 26	NS	00 0.5mM 0.1mM	32 31 31	NS
ampicillin	0mM 0.5mM 1.0Mm	36 33	0.05	00 0.5mM 0.1mM	37 35 35	NS
Erythromycin	0mM 0.5mM 1.0Mm	30 30 30	NS	00 0.5mM 0.1mM	32 32 32	NS
Clindamycin	0mM 0.5mM 1.0Mm	31 31 29	NS	00 0.5mM 0.1mM	34 33 32	NS
Doxycycline	0mM 0.5mM 1.0Mm	31 31 29	0.05	00 0.5mM 0.1mM	34 33 31	NS
Cephradine	0mM 0.5mM 1.0Mm	32 31 30	NS	00 0.5mM 0.1mM	32 31 30	NS

***MIC of amoxicillin following exposure to heavy metals in* S. aureus**

The minimum inhibitory concentration (MIC) of amoxicillin for S. aureus under chromium and amoxicillin co-exposure conditions was ascertained using the agar dilution method.

On the other hand, in the MHA plates supplemented with ≥0.125 µg/mL amoxicillin, no detectable CFU were found for unexposed S. aureus (SupplementaryFig.S1). For unexposed S. aureus, the MIC of amoxicillin was thus 0.06 µg/mL. Pre-exposure to a low dose of both chromium and amoxicillin increased the minimum inhibitory concentration (MIC) of amoxicillin for S. aureus by an overall 8-fold, while pre-exposure to chromium alone increased the MIC by 2 fold and amoxicillin alone increased the MIC by 4 fold when compared to control.

Antimicrobial susceptibility patterns of chromium pre-exposed S. aureus

Liquid broth medium supplemented with concentrations of 0.06 µg/mL, 0.125 µg/mL, 0.25 µg/mL, and 0.5 µg/mL were used to cultivate S. aureus following pre-exposure to either amoxicillin alone or in combination with chromium. Overall, the amoxicillin susceptibility patterns have shown that the size of the ZOI for amoxicillin was dramatically reduced when chromium and low concentration amoxicillin were exposed together.

***Antibiotic susceptibility pattern of cadmium pre-exposed* S. aureus**

The bacteria with changed minimum inhibitory concentration (MIC) were used to cultivate S. aureus on TSB medium, either with or without prior exposure to amoxicillin, cadmium, or both. These suspensions of the active ingredients were distributed on Mueller-Hinton Agar Media (Supplementary). In each of the several co-

exposure scenarios, there was a significant decrease in the zone of inhibition. The ZOI, for instance, was 32 mm for bacteria that had not been treated to amoxicillin, cadmium, or co-exposure conditions; it was 27 mm, 26 mm, and 25 mm, respectively.

***Amoxicillin susceptibility patterns and efflux activity of chromium or cadmium pre-exposed reference strain of* S. aureus**

The MIC of amoxicillin against the reference strain of S. aureus FDA 209 (ATCC #6538) after exposure to chromium or cadmium was ascertained using the agar dilution method. Amoxicillin's minimum inhibitory concentration (MIC) against S. aureus subspecies FDA 209 rose to 0.125 µg/mL after 48 hours and 0.25 µg/mL after 72 hours when exposed to chromium or cadmium. Additionally, the efflux assay showed that, in comparison to the unexposed control, the efflux of ethidium bromide was much higher in chromium or cadmium-preexposed S. aureus.

***Expression patterns of genes encoding efflux pumps and femX gene in* S. aureus**

Coexposure of amoxicillin to heavy metals such as cadmium and chromium modifies amoxicillin susceptibility and encourages the formation of amoxicillin-resistant S. aureus, as seen by antimicrobial susceptibility patterns.

DISCUSSION

Because of its alarming daily rise in antibiotic resistance, S. aureus is a major superbug in the healthcare industry. Notably, the possibility of bacterial co-exposure in water bodies was raised by the excessive use of antibiotics in cattle and the presence of heavy metals in untreated effluents from the leather industry.[18] In this work, we postulated that heavy metals could modify the degree of antibiotic susceptibility and promote the formation of S. aureus that is resistant to multiple drugs. Furthermore, this study's antimicrobial susceptibility profile showed that amoxicillin-resistant S. aureus can be driven by very low inhibitory concentrations of chromium or cadmium and amoxicillin pre-exposure. Emerging antibiotic resistance superbugs in environmental reservoirs may be caused by changes in the sensitivity of bacteria to antibiotics in pre-exposed chromium and cadmium salts.

TABLE 4

Concentration and purity of RNA of s.aureus and synthesized cDNA.

Condition	Sample	RNA concentration (ng/µl)	cDNA concentration (ng/µl)	Absorbance ratio (260nm/280nm)	Absorbance ratio (260nm/230nm)
48 hours pre-exposed s.aureus	Control	160.4	1500.1	1.82	2.15
		167.0	1762.3	1.75	2.07
		126.0	1165.3	1.83	2.23
		179.3	1352.3	1.81	2.18
		167.0	1262.0	1.82	2.22
		360.1	1240.9	1.84	2.23
		135.9	1219.2	1.83	2.21
		149.9	1297.5	1.84	2.24
		134.2	1226.0	1.82	2.19
Unexposed s.aureus	Control	235.9	1279.0	1.82	2.21
		240.3	1144.1	1.83	2.23
		284.0	1252.4	1.83	2.23
		270.9	1127.1	1.81	2.21
		236.8	1316.7	1.83	2.20
		158.9	1074.5	1.82	2.22

CONCLUSION

The impact of co-exposure to chromium or cadmium and low amoxicillin concentration on the emergence of S. aureus resistant to amoxicillin was underlined in this investigation. Through the use of a culture-based method, the antibiotic-resistant bacteria that emerged de novo was verified. It is necessary to do additional study to determine how the efflux pump contributes to the development of amoxicillin resistance in other bacterial populations. Studies should also focus on therapeutically useful antibiotics, such as semi-synthetic penicillins, which are used to treat Staphylococcus aureus.

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