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Prevalence, Antimicrobial Susceptibility and Genotypic Characterization of *Escherichia coli* Pathotypes Isolated from Children with Diarrhoea in Meru-Kenya

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Abstract

Introduction: There has been a global decrease in diarrhea infections in both adults and children due to the introduction of the Rotavirus vaccine and improved water, sanitation, and hand washing interventions. Low- and middle-income countries, however, continue recording high cases of diarrhea especially among children below five years where bacterial infections are one of the main causes. Particularly, *E. coli* has been isolated as the major bacterial etiological agent responsible for diarrhea among children in developing countries in Africa and Asia. There is also an increasing trend in antibiotic resistance in enteric pathogens against common and locally available prescribed drugs. This study aims to determine the prevalence and antimicrobial profile of *E. coli* as a causal agent of diarrhea among children in Meru. **Methodology:** A cross-sectional study design was conducted to recruit children with diarrhea seeking medical services at Meru Teaching and Referral Hospital. A total of 210 children were recruited for the study, and stool samples were collected from them for microbiological analysis. Multiplex PCR was done to detect *E. coli* pathotypes and ESBL genes. The disk diffusion method was used to determine the antimicrobial susceptibility of the *E. coli* pathotypes. **Results:** Of the 210 samples collected, 150 (71.4%) samples were positive for *E. coli* and 68 (32.4%) were positive for at least one virulence gene. EAEC was the most predominant pathotype at 23.53% followed by EPEC at 20.59% and ETEC at 14.71%. EHEC was the fourth common pathotype at 13.24% while EIEC prevalence was 11.76%. Isolates with co-infection were also detected with EPEC + ETEC being most common at 8.82% of the isolates followed by EPEC + EAEC

at 4.4% and ETEC + EAEC at 2.9%. The ages between 13 - 24 months were the most affected with DEC at 45.6% followed by the age group of 25 - 36 months at 23.6% while the least affected was the age groups of 49 - 60 months at 6%. The antimicrobial susceptibility profile of the DEC showed the highest resistance against Sulfamethoxazole/Trimethoprim at 88.2% followed by Amoxicillin/Clavulanic acid at 83.8% and Ciprofloxacillin at 38.2%. Resistance of DEC against Gentamicin was at 11.8%, and resistance against Meropenem was the lowest at 1.5%. All DEC isolates tested were susceptible to Tetracycline, and 95.6% were susceptible to Meropenem. Others were Gentamicin at 80.9%, Ciprofloxacin at 52.9% and Amoxicillin/Clavulanic acid at 16.2%. No susceptibility was observed towards Sulfamethoxazole/Trimethoprim in all the samples tested. The tested DEC pathotypes showed high carriage of ESBL with 63 (92.7%) turning positive for at least one ESBL gene. Generally, blaTEM was most common accounting for 43% while blaCTX-M accounted for 26% and blaSHV was 3%. 9% of the DEC isolates had both blaTEM and CTX-M while 3% of the isolates had all the three ESBL genes. **Conclusion:** This study reports predominance of Enteroagregative *E. coli*, high resistance to Sulfamethoxazole/Timethoprime and Amoxicillin/Clavulanic acid and ESBL carriage in DEC among the study population.

Keywords

Diarrhegenic *E. coli* (DEC), Pathotypes, Antimicrobial Resistance (AMR), Extended Spectrum Betalactamase (ESBL)

1. Introduction

Diarrhea is a symptom of an intestinal tract infection characterized by the passage of loose or watery stool more than normal for an individual and mostly three or more times a day [1]. Globally there is a reported decrease in diarrhea cases due to effective strategies including vaccination and improved hygiene practices. This is, however, not the case in poor communities where children continue to suffer the burden of diarrhea due to inadequate access to clean water for effective water sanitation and hand washing (WASH) interventions [2] [3]. Several studies have detected enteropathogens as the major cause of diarrhea in low- and middle-income countries especially in Africa, the most common being *Rotavirus, E. coli, Salmonella* and *Shigella* pathogens. Among the children *E. coli* continues to lead as the major causative agent [4] [5].

E. coli is a member of normal micro-flora in humans but some species such as EHEC, EPEC and EAEC have virulence factors that can cause intestinal infections [3]. Adults are capable of harboring DEC without developing disease unlike children who are highly affected. This poses a risk of cross-contamination between healthy adults and children. Kenya, like many other developing states, has in previous studies recorded a higher prevalence of DEC than developed countries such as Indonesia [6].

The burden of diarrhea is worsened by antibiotic resistance which remains a global threat to health. It has become harder to treat the emerging and growing number of infectious diseases such as diarrhea, pneumonia and tuberculosis. Antibiotic resistance can either occur naturally or due to misuse of antibiotics and can affect anyone regardless of age or geographical location [1]. Enteric *E. coli* pathotypes have been found to harbor resistance genes including the Extended Spectrum Beta-lactamase (ESBL) making them multi drug resistant (MDR) especially to third class generation antibiotics. ESBL encoding genes including *bla*TEM, *bla*SHV and *bla*CTX-M have been isolated in both healthy and diseased persons [7].

Standardized control and management of antibiotic resistance is one of the strategies put in place by WHO to fight the resistance through The Global Antimicrobial Resistance Surveillance System [8].

2. Statement of the Problem

Diarrhea deprives the body of water and salts in the case it lasts for several days making it responsible for more than half a million deaths in children annually and is the second leading infectious cause of death in children under the age of five years globally. The most common causes of infectious diarrhea include the *rotavirus, E. coli, Cryptosporidium* and *Shigella* species [1].

Globally, diarrhea infections among children below five years have been the cause of about 8% of all neonatal deaths. This is despite diarrhea being a treatable infection [9], a scenario that can be explained by the emergence of and global spread of antibiotic resistance [5].

Escherichia coli is among the bacterial strains that have been determined to be a major cause of diarrhea in children. These strains of *E. coli* have been isolated as a cause for previous diarrhea outbreaks in several parts of the world in the last decade [10] [11].

Despite a significant decline in child mortality caused by diarrhea in developing countries, this burden remains a reality especially in the poorest communities [2] [12] due to poor hygiene and sanitation practices [3]. The burden of diarrhea infections therefore poses a threat to the already ailing economic stability of households in low- and middle-income countries [13]. Diarrheagenic *E. coli* pathotypes among children under five years of age in Sub-Saharan Africa have been estimated to be at 15.6% [14], while in East Africa is at 27% [15].

Research studies conducted in various parts of Kenya have given the same results identifying *E. coli* pathotypes as a major etiological agent causing diarrhea, a reflection of many other low- and middle-income countries [16]. These pathotypes resulted in 5.85% of mortality cases in Kenya [8].

It was also established that the strains causing this enteric disease are gaining resistance to common chemotherapeutic drugs [5]. In this regard, *E. coli* strains isolated in Meru showed antimicrobial resistance and had a high prevalence ranging from 16.67% to 66.67%.

3. Justification

Diarrhea continues to be a major set-back in fighting high rates of child mortality in low- and middle-income countries. In order to effectively reduce the mortality rates in children, continuous detection of causative agents is important for proper treatment. Since many of the diarrhea cases are treated with antibiotics [16], it is important to also continually screen for the susceptibility of the common antibiotics to close the wide data gap in developing nations [17]. This research will therefore give concern to *E. coli* pathotypes in children below five years to help in better understanding their prevalence and susceptibility to available drugs for improved location-specific public health strategies and better treatment. To get a wider geographical data representation, Meru Teaching and Referral Hospital being a medical hub in Meru and its neighboring counties, was found to be a suitable study site. The results of this research will therefore be helpful to public health policy makers and medical service providers especially in Meru Teaching and Referral Hospital where, to the best of my knowledge, no such study has been done targeting the population that depends on this medical facility.

4. Materials and Methods

4.1. Study Site

Sample collection was done in the Meru Teaching and Referral Hospital (MeTRH) which serves both inpatients and outpatients.

MeTRH being the only national referral hospital in the eastern part of the country is a medical hub that serves not only the residents of Meru County but also of the neighboring counties of Tharaka Nithi, Isiolo, Nyeri and Laikipia most of which are still struggling with poor sanitation, inadequate and/or unsafe water.

4.2. Study Design

This was a cross-section study undertaken among children with diarrhea seeking medical attention at Meru Teaching and Referral Hospital.

4.3. Study Population

The study targeted children below five years of age with diarrhea seeking medical services in Meru Teaching and Referral Hospital, in-order to determine the prevalence and antimicrobial susceptibility of *E. coli* pathotypes as the enteric pathogen causing diarrhea among children.

4.3.1. Inclusion Criteria

- Children between 0 - 59 months of age.
- Children presenting with diarrhea (three or more episodes of loose/watery stool).
- Children whose assent was given by parents or guardians.

4.3.2. Exclusion Criteria

- Children with diarrhea who are already on antibiotic treatment.

- Children with diarrhea but with known underlying medical conditions.

Sample size Determination

The method explained by Taherdoost. [18] was used to determine the sample size for this study

$$N = P(100-P)Z^2/E^2$$

where:

N = the required sample size

P = the percentage estimate of occurrence of the desired character in the target population, 6.9% [19]

E = the percentage maximum error required, 5%

Z = the value of level of confidence required (Z=1.96 at a level of confidence of 95%)

$N = 0.069\ (1 - 0.069)1.96^2/0.05^2 = 98.71$

$N = 99$

The study was however able to collect additional samples up to a total number of 210 samples.

4.4. Sampling Method

A simple random sampling criterion was used whereby enrollment of participants to this study was done consecutively. All children below five years of age presenting with diarrhea were eligible to participate. A consent was sought from the parents and consenting participants were recruited to a total sample size of 210.

4.5. Specimen Collection

About 5 - 10 grams of stool sample was collected in a 30 mL screw cap container. In the case of the absence of stool, a rectal swab from each candidate was done. The samples were taken to the Meru Hospital laboratory for analysis.

4.6. Lab Procedure

The stool/anal swabs were directly streaked and cultured on MacConkey agar. Biochemicals tests: hydrogen sulfide production, motility and citrate utilization tests were done as confirmatory tests of *E. coli* isolates. A pure colony of presumptive *E. coli* isolates was inoculated in tryptic soy broth agar with 15% glycerol. The isolates were stored at −4˚C for further analysis.

Qiagen DNA extraction kit was used to extract DNA following the manufacturer's instructions. Multiplex PCR was done to amplify *eaeA* and *bfpA* for EPEC, *elt* and *stlA* for ETEC, *ial* for EIEC, CVD432 for EAE, *hlyA* and *eaeA* for EHEC. A 20.0 µl PCR reaction mixture contained 10.0 µl master mix (HotStarTaqPlus Master Mix, 2x), 0.4 µl primer, 7.6 µl nuclease-free water and 2.0 lysate. The reaction was ran with an initial inactivation of blocking antibody at 95˚C for 2 minutes, followed by 35 cycles of amplification (95˚C for 60 seconds, 61˚C for 60 seconds, 74˚C for 23 seconds and final extension at 74˚C for 23 minutes). Nuclease-free water was used as negative control and ATCC 25922 as positive control.

The amplified genes were ran on 2.5% agarose gel and viewed under UV camera box. To detect ESBL genes, Multiplex PCR was done to amplify *blaSHV, blaTEM and blaCTX-M* genes. 20.0 µl PCR reaction mixture contained 10.0 µl master mix (HotStarTaqPlus Master Mix, 2x), 0.36 µl primer, 7.64 µl nuclease-free water and 2.0 lysate. The reaction was ran with an initial denaturation at 95˚C for 5minutes, followed by 35 cycles of amplification (94˚C for 30 seconds, 58˚C for 30 seconds, 72˚C for 45 seconds and final extension at 72˚C for 10 minutes). Nuclease-free water was used as negative control and ATCC 25922 as positive control. The amplified genes were ran on 2.5% agarose gel and viewed under UV camera box.

The standard disc diffusion method was used to test the antimicrobial susceptibility of all the DEC isolates against the following antibiotics: Amoxycillin/Clavulanic acid (AMC 20 µg), Meropenem (MEM 10 µg), Gentamicin (GEN 10 µg), Ciprofloxacin (CIP 5 µg), sulphamethoxazole/trimethoprim (SXT 25 µg) and tetracycline (TE 30 µg). The diameter of zones of Inhibition (ZOH) were measured in millimeter (mm) using a ruler and interpretations made according to CLSI 2024 guidelines.

4.7. Ethical Consideration

Approval for the study was obtained from the ethical committee of Jomo Kenyatta University Institutional Review Board (Ref: JKU 2/4/896B). Research permit was sought from NACOSTI (REF NO. 775382) and Meru teaching and referral Hospital. A signed consent form was obtained from parents before sample collection from children.

5. Results

Of the 210 samples collected, 150 (71.4%) samples were positive for *E. coli* and 68 (32.4%) were positive for at least one virulence gene. Of the 68 DEC *E. coli* isolates 38 (55.8%) were male while female candidates were 30 (44.12%). The ages between 13 - 24 months were the most infected with DEC at 45.6% followed by the age group of 25 - 36 months at 23.6% while the least affected was the age groups of 49 - 60 months at 6%. EAEC was the most predominant pathotype at 23.53% followed by EPEC at 20.59% and ETEC at 14.71%. EHEC was the fourth common pathotype at 13.24% while EIEC prevalence was 11.76%. Isolates with co-infection were also detected with EPEC + ETEC being most common at 8.82% of the isolates followed by EPEC + EAEC at 4.41% and ETEC + EAEC at 2.94%.

The antimicrobial susceptibility profile of the DEC showed the highest resistance against Sulfamethoxazole/Trimethoprime at 88.2% followed by Amoxicillin/Clavulanic acid at 83.8% and Ciprofloxacillin at 38.2%. Resistance of DEC against Gentamicin was at 11.8%, and resistance against Meropenem was the lowest at 1.5%. All DEC isolates tested were susceptible to Tetracycline and 95.6% of the isolates were susceptible to Meropenem. Others were Gentamicin at 80.9%, Ciprofloxacin at 52.9% and Amoxicillin/Clavulanic acid at 16.2%. No susceptibility was observed toward Sulfamethoxazole/Trimethoprime in all the samples

tested. The study also reports MDR with twenty isolates showing resistance to three different drugs while 3 isolates were resistant to four different drugs tested. EPEC is reported with most MDR while EHEC was the least pathotype with MDR.

The tested DEC pathotypes showed a high prevalence of ESBL with 63 (92.7%) turning positive for at least one ESBL gene. Generally, blaTEM was most common accounting for 43% while blaCTX-M accounted for 26% and blaSHV was 3%. 9% of the DEC isolates had both blaTEM and CTX-M while 3% of the isolates had all the three ESBL genes.

6. Discussion

There is lack of real time data on DEC infections especially in Africa, a situation that is linked to poor diagnostic test systems [20]. However, the available data shows DEC is among the major causative agent responsible for diarrhea illness among children. In the current study, the prevalence of DEC in Meru is reported at 32.4% similar to a recent study conducted in Nakuru which reported the prevalence at 34.2% [21]. The reported prevalence in Meru is however slightly higher than the reported prevalence in Nairobi and in East Africa both at 27% [15] [22] and much higher than other studies which reported as low as 15% [23] However, the current study shows a higher prevalence than the prevalence in sub-Sahara Africa region which was reported at 15.6% [14]. The burden may vary due to differences in study location, inclusion criteria of the study population and lab procedures. In the current study, the male dominated with a higher prevalence of DEC at 55.88% while female represented 44.12%.

Table 1 shows the pathotypic distribution of DEC in this study where EAEC was the most common pathotype (23.53%) consistent with recent studies conducted in Kenya and Ethiopia [22] [24]. This pathotype is commonly associated with acute, prolonged and persistence diarrhea in children, patients with HIV infections and acute travelers' diarrhea to travelers in low- and middle-income countries. EPEC was the second most prevalent pathotype accounting for 20.59% of the all the pathotypes detected. Our findings are in line with [25] where EPEC was reported as the second most prevalent pathotype. Although the study only found atypical EPEC pathotype in their study, the current study contradicts his findings by detecting both typical and atypical EPEC. Other studies have reported lower percentage of EPEC accounting for 10% while others have reported very high percentage of up to 51% among children in Kenya [22] [26] indicating its epidemiology is changing from time to time and place to place. The current study also reports detection of mixed infections accounting for 16.18% of the DEC. These included the three cases with EAEC + EPEC, six cases with EPEC + ETEC and two cases with ETEC + EAEC. The presence of theses co-infections in our study concurs with recent reports in other studies. Similar to this study, EPEC + EAEC pathotype have been reported in Brazil [27], and EPEC + ETEC in India [28]. Presence of such hybrid pathotypes are a concern in fight against DEC as they have been linked to multi-drug resistance [29]. ETEC was the fourth common pathotype detected at

14.71% consistent with other studies in China at 14.8% [30] and Nigeria 17.3% [31]. EHEC followed at 13.24% in current study consistent with findings in Nairobi 19% [22] This pathotype is typically a zoonotic food-borne pathotype causing bloody diarrhea and may also lead to kidney failure [32]. Children in their explorative stage and those exposed to interactions with domestic animals are more likely to get infected with this pathotype [33] [34]. The current study however found no statistically significant association between age group and pathotype (P value 0.332). EIEC was the least detected DEC pathotype accounting for 11.76%.

Table 1. Prevalence of *E. coli* pathotypes in different age groups (in Months) and gender.

Gender	EAEC	EAEC + EPEC	EHEC	EIEC	EPEC	EPEC + ETEC	ETEC	ETEC + EAEC	Total	%
F	6	1	4	4	7	3	4	1	30	44.12
M	10	2	5	4	7	3	6	1	38	55.88
Age										
0 - 12	4	0	0	0	4	0	0	0	8	11.76
13 - 24	5	1	6	4	4	3	6	2	31	45.59
25 - 36	5	0	2	3	3	1	2	0	16	23.53
37 - 48	0	2	1	1	2	1	2	0	9	13.24
49 - 60	2	0	0	0	1	1	0	0	4	5.88

The present study reveals high susceptibility towards Gentamicin and Meropenem at 80.9% and 95.6% respectively as shown in **Table 2** concurring with another study done in Kisumu showing susceptibility of Gentamicin at 80.8% and Meropenem at 84.6%. The two studies however differ in the case of tetracycline where the current study reports 100% sensitivity while the latter reports high resistance of the drug at 100% [35]. Similarly, [24] reported a high resistance to Tetracycline at 91.3% in a study conducted in central Ethiopia. However, high resistance is revealed against SXT (88.2%) deferring with Zelelie whose study in central Ethiopia reported low resistance at 42.6%. This study is comparable with reports from the study in Kisumu which have also reported high resistance to AMC at 80.8% [35].

Hybrid strains have been revealed to show high resistance towards tested drugs with most strains with 100% resistance being hybrid strains as shown in **Table 3**.

Table 2. Antimicrobial susceptibility of tested drugs.

Antibiotic	%R	%I	%S	%R 95% C.I.	%S 95% C.I.
Amoxicillin/Clavulanic acid	83.82	0	16.17	72.5 - 91.3	8.7 - 27.5
Meropenem	1.47	2.94	95.58	0.1 - 9.0	86.8 - 98.9
Gentamicin	11.76	7.35	80.88	5.6 - 22.4	69.2 - 89.0
Ciprofloxacin	38.23	8.82	52.94	27.0 - 50.9	40.5 - 65.0

Continued

Trimethoprim/Sulfamethoxazole	88.23	11.76	0	77.6 - 94.4	0.0 - 6.7
Tetracycline	0	0	100	0.0 - 6.7	93.3 - 100
Key: R—Resistance; S—Susceptible; I—Intermediate;					

Table 3. Drug resistance profile across *E. coli* pathotypes.

Pathotype	Number	GEN %	SXT %	MEM %	TCY %	CIP %	AMC %
ETEC + EAEC	02	0.00	100	0	0	50.0	100
ETEC	10	10.0	90.0	10	0	60.0	80.0
EPEC + ETEC	06	16.7	83.3	0	0	16.7	100
EPEC	14	07.1	92.9	0	0	50.0	92.9
EIEC	08	12.5	100	0	0	25.0	75.0
EHEC	09	11.1	88.9	0	0	22.2	66.7
EAEC + EPEC	03	0.00	100	0	0	100	100
EAEC	16	18.8	75.0	0	0	25.0	81.3

Similar to other studies carried out in other parts of Kenya and Iran [22] [36] this study reports prevalence of MDR among DEC from children. This study reveals a prevalence of 33.82% of MDR lower than findings by [37] at 92.3%. This study only found 20 isolates being resistant to three different drugs while 3 isolates were resistant to four different drugs tested. **Table 3** and **Table 4** show the sensitivity results of different pathotypes towards the tested drugs where EPEC had the highest prevalence of MDR and EHEC having the least while a study done in Ethiopia reported EAEC as the pathotype with highest prevalence of MDR [24]. The current study did not report any statistical significance association between pathotype and esbl status (P value 0.332, Chi sqr test statistic 30.66, egree of freedom = 28) blaTEM was the predominant beta-lactamase gene as shown in **Table 5** and **Table 6**, similar to another study in Addis Ababa [38] though in current study the prevalence was lower at 52.9% while in Addis Ababa was at 80%. Similarly, blaCTX-M was the second most predominant gene though again at lower percentage of 36.8% compared to 73% in Addis Ababa. This study however contradicts some other studies such as the one conducted in Tanzania and Ethiopia [39], [40] which reported blaCTX-M as the predominant gene. The discrepancy in carriage of ESBL in DEC could have resulted from differences in antimicrobial stewardship in the study regions. Children's environment and especially those in contact with livestock have been found to be at a higher risk of contracting ESBL producing DEC [40]. Studies that have looked at phenotypic proportion of ESBL-producing DEC have reported much lower prevalence of as low as 8.5% [40]. This study reports EAEC having the highest carriage of ESBL genes similar to [24] and contradicting with a study in Nairobi [22] while ETEC + EAEC had the least in the current study.

Table 4. Susceptible drug across *E. coli* pathotypes.

Pathotype	Number	GEN %	SXT %	MEM %	TCY %	CIP %	AMC %
EAEC	16	81.3	0	100	100	68.8	18.8
EAEC + EPEC	03	100	0	100	100	0.0	0.0
EHEC	09	66.7	0	88.9	100	77.8	33.3
EIEC	08	75.0	0	100	100	50.0	25.0
EPEC	14	92.9	0	92.9	100	50.0	07.1
EPEC + ETEC	06	83.3	0	100	100	66.7	0.0
ETEC	10	70.0	0	90.0	100	20.0	20.0
ETEC + EAEC	02	100	0	100	100	50.0	0.0

Table 5. Carriage of ESBL genes in *E. coli* pathotypes.

Pathotype	Number	(%)	Negative	CTX-M	SHV	SHV + TEM + CTX-M	TEM	TEM + CTX
EAEC	16	23.52	1	5	1	0	9	0
EAEC + EPEC	03	04.41	0	2	0	0	1	0
EHEC	09	13.23	2	3	0	0	4	0
EIEC	08	11.76	3	1	0	2	0	2
EPEC	14	20.58	3	2	1	0	7	1
EPEC + ETEC	06	08.82	0	1	0	0	4	1
ETEC	10	14.70	2	4	0	0	3	1
ETEC + EAEC	02	02.94	0	0	0	0	1	1

Table 6. Distribution of ESBL genes in gender and age (months).

Gender	CTX	SHV	SHV + TEM + CTX	TEM	TEM + CTX	Total	Percentage
F	7	1	2	13	3	26	45.61%
M	11	1	0	16	3	31	54.39%
AGE							
0 - 12	0	0	0	6	0	6	10.53%
13 - 24	6	2	1	11	5	25	43.86%
25 - 36	9	0	1	5	1	16	28.07%
37 - 48	2	0	0	4	0	6	10.53%
49 - 60	1	0	0	3	0	4	7.02%

7. Conclusions

The prevalence of pathogenic *E. coli* in Meru among children is high as in the case of many regions in developing nations with pathotypes being distributed across all age groups of the study population. The study confirms constant levels of high resistance to commonly prescribed antimicrobials. There is a very high prevalence

of ESBL producing *E. coli* pathotypes a situation that undermines the fight against diarrhea in developing countries.

A research on other causative agents is recommended in Meru among children to define all other causes and co-infections of diarrhea in the region. The study recommends antimicrobial susceptibility testing in routine laboratory tests and a change of treatment from commonly prescribed antibiotics to more effective ones. Factors attributing to the high prevalence and spread of ESBL producing pathogens among children need to be investigated as well as the presence of ESBL in non-pathogenic bacteria.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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