

Extended-Spectrum Beta-Lactamase and Metallo-Beta-Lactamase Production among *Escherichia coli* and *Klebsiella pneumoniae* Strains from Urine of Pregnant Women in Afikpo, Ebonyi State

ABSTRACT

Aim: The aim of this study was to determine the occurrence of Extended-spectrum beta-lactamase (ESBL) and Metallo-beta-lactamase (MBL) among *Escherichia coli* and *Klebsiella pneumoniae* strains from pregnant women attending Mater Misericordia Hospital Afikpo, Ebonyi state, Nigeria.

Study Design: This is a laboratory based prospective study carried out on pregnant women suspected of having urinary tract infection and was requested to undergo diagnosis at microbiology laboratory of the hospital.

Place and Duration of Study: The study was conducted in the Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Afikpo, Ebonyi State, Nigeria from October, 2022 to January, 2023.

Methodology: Clean-catch midstream urine samples were collected from 206 pregnant women suspected of having urinary tract infection and were requested to undergo medical diagnosis at microbiology laboratory of the hospital. The urine samples were processed following standard microbiological procedure. Antimicrobial susceptibility testing was determined using the disc diffusion method, while ESBL phenotypes were determined by the Double-Disc Synergy Test (DDST). Disc potentiation test was performed to check for MBL production.

Results: Out of the 206 urine samples processed, 24 (11.7 %) *E. coli* and 12 (5.8 %) *K. pneumoniae* were isolated. The antimicrobial susceptibility of the isolates recorded a 100 % resistance with Amoxicillin/Clavulanic acid and Cotrimoxazole. The Gram-negative isolates showed a high sensitivity of 100 % to Netillin, Meropenem and Ofloxacin. Overall, 35 (97.2 %) multidrug resistance (MDR) was observed of the bacteria isolates. A total of 9 (37.5 %) *E. coli* and 4 (33.3 %) *K. pneumoniae* was found positive for ESBL production whereas, 5 (20.8 %) *E. coli* and 2 (16.7 %) *K. pneumoniae* were MBL positive.

Conclusion: The level of drug resistance in this study underscores the need for regular surveillance for effective management of urinary tract infection in pregnancy.

Keywords: *Escherichia coli*, *K. pneumoniae*, Extended-Spectrum Beta-Lactamase, Metallo-beta-lactamase Meropenem.

1. INTRODUCTION

Urinary tract infection (UTI) is a health problem that is commonly associated with pregnancy [1]. Pregnant women are at a high risk of developing UTI due to physiological adaptations and anatomical changes during pregnancy [2, 3].

Urinary tract infections in pregnancy may present as asymptomatic bacteriuria, acute cystitis (bladder infection) or pyelonephritis (kidney infection) [4]. Asymptomatic bacteriuria occurs

in about 2 % to 10 % of all pregnancies and if untreated may develop acute cystitis and pyelonephritis in up to 30 % and 50 % of mothers [1, 4, 5]. Untreated bacteriuria has been associated with various adverse health outcomes in pregnancy such as low birth weight, preterm birth, sepsis, septic shock, anaemia and perinatal mortality [3, 4, 6].

Escherichia coli is the most common uropathogen associated with asymptomatic bacteriuria in approximately 80 % of cases; others include *Klebsiella pneumoniae*, *Enterobacter* species, *Proteus*, *Pseudomonas* and *Staphylococcus saprophyticus* [2, 3, 4, 7].

Antimicrobial resistance of uropathogens causing UTIs have shown steady increase over the past years. In a recent surveillance report published by WHO, *E. coli* and *K. pneumoniae* was found to be the most resistant pathogens causing UTIs [8]. Urinary tract infections caused by extended-spectrum beta-lactamase producing strains are increasing worldwide with increased severity in developing countries [9]. Infections caused by extended-spectrum producing uropathogens are difficult to treat because such strains are multidrug resistant, thereby limiting treatment options [10]. According to Osman et al. [11], mortality due to drug resistant infections are projected to increase from 700,000 to 10 million annually, with concomitant high cost of treatment of US \$100 trillion dollars worldwide by 2050. It is of concern that there is dearth of information regarding antimicrobial resistance in developing countries like Nigeria. Lack of adequate surveillance data fuel treatment failures reported in patients [12].

The increasing resistance against extended spectrum cephalosporins means that treatment of UTI has to rely on carbapenems often referred to as ‘drug of last resort’. Carbapenems are the drug of choice for the treatment of infections caused by ESBL producing bacteria [13, 14]. Unfortunately, carbapenem resistance due to metallo-beta-lactamase (MBL) production have been reported in several studies worldwide [15, 16].

Although, a number of studies in Nigeria have reported the prevalence of ESBL production and carbapenem resistance among pathogens causing UTI [12, 15], there is little or no data on the burden of ESBL and MBL production among uropathogenic bacteria causing UTI among pregnant women. The continued surveillance of drug resistance among ESBL and MBL producing uropathogens will help in formulating necessary policies to reduce the incidence of drug resistance and poor treatment outcomes. In this study, we determined the occurrence of ESBL and MBL among *E. coli* and *K. pneumoniae* isolated from urine of pregnant women attending Mata Misericordia Hospital, Afikpo, Ebonyi State, Nigeria.

2. METHODOLOGY

2.1 Study Design and Sample Collection:

This is a laboratory based prospective study conducted in the Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Afikpo, Ebonyi State, Nigeria from October, 2022 to January, 2023. Mid-stream urine (MSU) samples was collected from 206 pregnant women attending the antenatal clinic of Mater Misericordia Hospital, Afikpo, Ebonyi State. The pregnant women included in the study were those suspected of having urinary tract infection and was requested to undergo diagnosis at microbiology laboratory of the hospital.

2.2 Laboratory Analysis:

All the mid-stream urine samples were processed within 1 hour by centrifugation. The resultant precipitate was cultured on Cysteine Lactose Electrolyte-Deficient (CLED) agar and

McConkey agar (MCA) using streak plate and routinely incubated at 37°C for 24 hours under aerobic conditions.

2.3 Identification of Bacteria:

The bacteria was identified by their colony morphology, Gram staining characters, motility test, indole test, citrate utilization test, urease production test, Triple sugar iron agar test, methyl red test and Voges-Proskauer test [17].

2.4 Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was performed using Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [18]. Pure and identical colonies of 24-hour growth of the test organisms were suspended in a tube with 5 ml sterile physiological saline to get bacterial inoculums equivalent to 0.5 McFarland standards. A sterile cotton swab was dipped into the standardized inoculum and rotated across the wall of the tube to avoid excess fluid and was evenly inoculated on the surface of Muller-Hinton agar (MHA) (HiMedia, India). The inoculated plates was allowed to stand for about 5 minutes for absorption of excess moisture. Using sterile forceps, antimicrobial impregnated discs were placed on the media and incubated at 37 °C for 24 hours. The zones of inhibition was interpreted as sensitive, intermediate and resistant. The following antibiotics were tested; Ceftriaxone (CTR, 30 µg), Ceftazidime (CEF, 30 µg), Amoxicillin/Clavulanic acid (AMC, 30 µg), Gentamicin (GEN, 10 µg), Tetracycline (TE, 30 µg), Netillin (Netilmicin sulphate, NET, 30 µg), Co-Trimoxazole (Sulpha/Trimethoprim, COT, 25 µg), Levofloxacin (LE, 5 µg), Ofloxacin (OF, 5 µg), Meropenem (MER, 10 µg), Imipenem (IMP, 10 µg). Resistance shown to two or more antibiotics of different classes was considered multidrug resistance (MDR) [19].

2.5 Extended-Spectrum Beta-Lactamase (ESBL) Detection

The isolates with diameter of zone of inhibition of ≤ 25 mm for ceftriaxone and ≤ 22 mm for ceftazidime was verified for ESBL production by the Double Disc Synergy Test (DDST) on Mueller-Hinton agar using third generation cephalosporin's (Ceftriaxone [30 µg] and Ceftazidime [30 µg]) with a beta lactamase inhibitor (Amoxicillin + Clavulanic acid [30 µg]). The organism to be tested was spread onto Mueller-Hinton agar plate using similar procedures as in antimicrobial susceptibility testing. A disc of ceftriaxone (30 µg) and ceftazidime (30 µg) and amoxicillin + clavulanic acid (30 µg/10 µg) was placed 20 mm apart, centre to centre on Mueller-Hinton agar plate and incubated overnight at 37 °C for 24 hours. A zone difference greater than or equal to 5 mm around ceftriaxone or ceftazidime and ceftazidime + clavulanic acid is interpreted as ESBL positive strain [20].

2.6 Metallo-Beta-lactamase (MBL) Detection

All the isolates resistant to imipenem and/or meropenem was tested for MBL production by disc potentiating test with ethylenediaminetetraacetate (EDTA) impregnated imipenem and meropenem discs. A 0.5 M EDTA solution was sterilized by autoclaving. Standardized test organisms (as in antimicrobial susceptibility testing) were inoculated onto plates of Muller-Hinton agar. Two meropenem (10 µg) discs and two imipenem (10 µg) discs were placed on the inoculated plates 20 mm apart, centre to centre on Mueller-Hinton agar plate and 5 µl of EDTA solution was added to one meropenem disc and one imipenem disc. The plates were incubated overnight at 37 °C for 24 hours. A zone difference greater than or equal to 7 mm around meropenem and meropenem + EDTA disc and imipenem and imipenem + EDTA disc is recorded as MBL positive strain [21].

3. RESULTS

Of the total 206 urine samples processed, 24 (11.7 %) *E. coli* and 12 (5.8 %) *K. pneumoniae* were isolated. Overall prevalence of *E. coli* and *K. pneumoniae* in this study was 36 (17.5 %) (Table 1).

Table 1: Prevalence of *Escherichia coli* and *Klebsiella pneumoniae* isolates obtained from urine samples of pregnant women

Organism	Number of urine samples	Frequency	Percentage (%) prevalence
<i>E. coli</i>	206	24	11.7
<i>K. pneumoniae</i>	206	12	5.8
Total	206*	36	17.5

*Total urine samples analysed

In this study, the entire *E. coli* isolates were resistant to amoxicillin/clavulanic acid 24 (100 %) and cotrimoxazole (sulpha/trimethoprim) 24 (100 %) followed by ceftriaxone 15 (62.5 %). On the other hand, they showed 100 % sensitivity to netillin, meropenem and ofloxacin respectively. High sensitivity was also observed with levofloxacin 22 (91.7 %), gentamicin 21 (87.5 %), imipenem 20 (83.3 %) and ceftazidime 14 (58.3 %). *K. pneumoniae* showed 100 % resistance to cotrimoxazole (sulpha/trimethoprim) and amoxicillin/clavulanic acid, whereas 91.7 %, 66.7 % and 58.3 % resistance was observed against ceftriaxone, tetracycline and gentamicin respectively. The *K. pneumoniae* isolates were sensitive to meropenem, imipenem, levofloxacin, netillin and ofloxacin (100,100, 100, 75, and 66.7 % respectively) Table 2.

Table 2: Antimicrobial susceptibility pattern of bacterial isolates from urine samples of pregnant women

Isolates	Pattern	Antibiotic (%)										
		CTR (30 µg)	CEF (30 µg)	AMC (30 µg)	GE (10 µg)	TE (30 µg)	NET (30 µg)	COT (25 µg)	LE (5 µg)	OF (5 µg)	MER (10 µg)	IMP (10 µg)
<i>E. coli</i> (n = 24)	S	7 (29.2)	14(58.3)	-	21(87.5)	4(16.7)	24(100)	-	22(91.7)	24(100)	24(100)	20(83.3)
	I	2 (8.3)	2(8.3)	-	-	12(50.0)	-	-	2(8.3)	-	-	-
	R	15(62.5)	8(33.3)	24(100)	3(12.5)	8(33.3)	-	24(100)	-	-	-	4(16.7)
<i>K. pneumoniae</i> (n = 12)	S	-	6(50.0)	-	4(33.3)	-	9(75.0)	-	12(100)	8(66.7)	12(100)	12(100)
	I	1 (8.3)	-	-	1(8.3)	4(33.3)	2(16.7)	-	-	3(25.0)	-	-
	R	11(91.7)	6(50)	12(100)	7(58.3)	8(66.7)	1(8.3)	12(100)	-	1(8.3)	-	-

CTR = Ceftriaxone, CEF = Ceftazidime, AMC = Amoxyclav (Amoxicillin/Clavulanic acid), GEN = Gentamicin, TE = Tetracycline, NET = Netillin (Netilmicin sulphate), COT = Cotrimoxazole (Sulpha/trimethoprim), LE = Levofloxacin, OF = Ofloxacin, MER = Meropenem, IMP = Imipenem, % = Percentage, n = Number, µg = Microgram, S = Sensitive, I = Intermediate, R = Resistance.

Overall, 35 (97.2 %) multidrug resistance (resistance to two or more antimicrobials agents) was observed of the bacteria isolates (Table 3).

The overall proportion of ESBL production was at 13 (36.1 %) whereas the proportion of MBL production was at 7 (19.4 %) (Table 4). Specifically, 9 (37.5 %) *E. coli* and 4 (33.3 %) *K. pneumoniae* was found positive for ESBL production. The positive MBL production was 5 (20.8 %) and 2 (16.7 %) for *E. coli* and *K. pneumoniae* respectively.

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Table 3: Multidrug resistance pattern of *E. coli* and *K. pneumoniae* isolates from urine samples of pregnant women

Isolates	Total	Frequency (%)					Total MDR
		R ₂	R ₃	R ₄	R ₅	R ₆ and above	
<i>E. coli</i>	24 (66.7)	2(8.7)	12(52.2)	3(13.0)	5(21.7)	1(4.3)	23 (95.8)
<i>K. pneumoniae</i>	12 (33.3)	-	1(8.3)	4(33.3)	4(33.3)	3(25.0)	12 (100)
Total	36 (100)	2(5.7)	13(37.1)	7(20.0)	9(25.7)	4(11.4)	35 (97.2)

R₂ = Resistance to two antibiotics, R₃ = Resistance to three antibiotics, R₄ = Resistance to four antibiotics, R₅ = Resistance to five antibiotics, R₆ and above + Resistance to six and more antibiotics, MDR = Multidrug resistance, % = Percentage.

Table 4: Proportion of ESBL and MBL production among the bacterial isolates

Bacteria	Number	%	ESBL Production n (%)	Non-ESBL Production n (%)	MBL Production n (%)	Non-MBL Production n (%)
<i>E. coli</i>	24	66.7	9 (37.5)	15 (62.5)	5 (20.8)	19 (79.2)
<i>K. pneumoniae</i>	12	33.3	4 (33.3)	8 (66.7)	2 (16.7)	10 (83.3)
Total	36	100	13 (36.1)	23 (63.9)	7 (19.4)	29 (80.6)

ESBL = Extended spectrum beta-lactamase, MBL = Metallo-beta-lactamase, % = Percentage, n = Number

4. DISCUSSION

Urinary tract infection in pregnancy has been identified as the common cause of hospital visit among pregnant women in different settings [22]. In the present study, a total of 206 urine samples were processed out of which *E. coli* 24 (11.7 %) and *K. pneumoniae* 12 (5.8 %) were isolated. The overall prevalence of the isolates was 36 (17.5 %). This finding is low compared to studies conducted in South-Western Uganda by Johnson et al [23] who reported 40 (28.78 %) *E. coli* and 52 (37.41 %) *K. pneumoniae* among pregnant women with an overall prevalence of UTI of 35 %. In Edna Adan Hospital, Hargeisa, Somaliland, Hussein et al [22] reported a higher prevalence of 36 (45.6 %) and 16 (20.3 %) for *E. coli* and *K. pneumoniae* respectively. Dube et al. [7] reported *E. coli* (27 %) and *K. pneumoniae* (20.7 %) as the most common organisms causing UTI in pregnant women in Abdullah Bin Omran Hospital, United Arab Emirate (UAE). Another study in UAE isolated *E. coli* (30.9 %) and *K. pneumoniae* (13.1 %) [24]. In Hargeisa Group Hospital, Hargeisa, Somaliland, Ali et al. [1] reported *E. coli* (61.2 %) as the most predominant bacteria followed by *K. pneumoniae* (12.2 %). In Western Ethiopia, *E. coli* (53.8 %) was the predominant bacterial isolate followed by *K. pneumoniae* (17.95 %) [25].

The overall prevalence of 17.5 % observed in this study is slightly higher than the 16.4 %, 16.8 %, 15.7 %, 15.8 % and 15.0 % reported in Hargeisa, Somaliland [1]; Mwanza City in Tanzania [26]; Nairobi, Kenya [27]; Kano, Northern, Nigeria [28]; and Bangalore, India [29] respectively. A higher prevalence of 18.7 % was reported in Ambo Central Ethiopia [30]; in Southern Nigeria 25.3 % [31], in Derna City Libya 49.3 % [32]; in Ismailia, Egypt 29.0 % [33]; in Benin City, Nigeria 21.0 % [34]; in Saudi Arabia 53.5 % [35] and in Nepal 37.8 % [36]. The reasons for these variations in prevalence is not far from differences in sample size, social habits of the community and the standard of personal hygiene [37].

Consistent with the present study, several studies have earlier implicated *E. coli* followed by *K. pneumoniae* as the most commonly isolated uropathogen causing UTI [1, 22, 25, 38]. This could be explained by the increased susceptibility of uroepithelium to colonization by coliforms [7] and their ability to produce several virulence factors [39]. In contrast, Johnson et al [23], found *K. pneumoniae* as the dominant pathogen causing UTI followed by *E. coli* in their study.

Antimicrobial resistance of *E. coli* and *K. pneumoniae* in this study compared to earlier studies reports. The Gram negative isolates were entirely resistant to amoxicillin/clavulanic acid (100 %) and cotrimoxazole (Sulpha/trimethoprim) (100 %). The *K. pneumoniae* isolates showed a resistance pattern of 91.7 % > 66.7 % > 58.3 % to ceftriazone, tetracycline and gentamicin respectively. Similar antimicrobial susceptibility pattern was reported by Melaku [19] and Mohammed and Abass [40]. Johnson et al. [23] reported 95.0 % resistance to amoxicillin/clavulanic acid. Ali et al. [1] observed the organisms to be resistant to tetracycline (71.4 %), trimethoprim-sulfamethoxazole (57.1 %) and amoxicillin/clavulanic acid (55.1 %). Bahati et al. [41] and Derese et al. [42] observed a high resistance of 72.9 % and 73.7 % to amoxicillin/clavulanic acid and tetracycline respectively. The level of resistance observed against amoxicillin/clavulanic acid is worrisome because the drug is prescribed in this setting in the management of UTIs. It has also been approved in some settings as an alternative drug in the treatment of UTIs in pregnant women [43]. The level of resistance to antibiotics observed in this study is attributable to indiscriminate and misuse of antibiotics, in addition to self-medication and use of antimicrobial drugs without prescription which is almost common in Nigeria.

However, the *E. coli* isolates showed very high sensitivity to meropenem (100 %), ofloxacin (100 %), netillin (100 %), levofloxacin (91.7 %), gentamicin (87.5 %) and imipenem (83.3 %); whereas, the *K. pneumoniae* isolates were entirely sensitive to meropenem (100 %), imipenem (100 %), levofloxacin (100 %) followed by netillin (75.0 %) and ofloxacin (66.7 %). Our finding is comparable to studies done in Western Ethiopia where susceptibility of *E. coli* and *K. pneumoniae* to meropenem were found to be 95.2 % and 100 % respectively [25]. In South Western Uganda, Johnson et al. [23] reported a sensitivity of 82.9 % to gentamicin. In Hargeisa, Somaliland, Ali et al. [1] found that most the Gram negative isolates were sensitive to meropenem (95.9 %) and gentamicin (75.5 %). In Addis Ababa, Ethiopia, Wabe et al. [44] reported a high sensitivity to meropenem (75.2 %) and gentamicin (85.2 %). In Southern Nigeria, Akpan et al. [45] observed sensitivity to gentamicin (53-100 %) and imipenem (67-93 %).

In this study, multi-drug resistance (MDR) was observed to be 97.2 %. This finding is higher than 60.7 % MDR reported against Gram negative uropathogens by Abu et al. [25]. In Dessie, North-East Ethiopia, Tadesse et al. [37] reported MDR of 72.4 %. Ali et al. [1] reported 85.5 % MDR in Somaliland whereas 73.0 %, was seen in Mekella by Wabe et al. [44]. In Eastern Uganda, Nteziyaremye et al. [46] reported 77.5 % MDR. Our finding is lower than that of earlier studies done in South-South, Nigeria (100 %) [45]. Similarly, Johnson et al. [23] and Derese et al. [42] observed 100 % MDR in their respective studies.

The presence of ESBL producers among the Gram negative isolates in our study was 13 (36.1 %) with *E. coli* 9 (37.5 %) and *K. pneumoniae* 4 (33.3 %) respectively. This was higher than 19 % reported by Onyango et al. [27] in Kenya and 18 % by Sekikubo et al. [47] at Mulago. In Nigeria, Onwuezobe and Orok [48] found a lower 6 (20.0 %) ESBL producers. Similarly, 15.8 % of the Gram negative isolates were found to ESBL producers by Melaku [19] in his study. Hussaein et al. [22] reported 38.8 % and 31.3 % level of ESBL production by *E. coli* and *K. pneumoniae* respectively. This finding varied slightly with the 37.5 % and 33.3 % seen in our study. A systemic review on the prevalence of ESBL production among Gram negative bacteria in Nigeria, found a prevalence range from 7.9 % to 65 % in North-Central Nigeria, North-East Nigeria (16.7 % to 82.3 %); North-West Nigeria (12.8 % to 41.2 %), South-East Nigeria (8.1 % to 74.3 %), South-South Nigeria (8.9 % to 47.1 %) and South-West Nigeria (7.5 % to 76.9 %) [12]. With the findings of our study and earlier reports, there is an indication of widespread production of ESBL among Gram negative bacteria in Nigeria [12]. ESBL production among uropathogenic bacteria is a global problem that cannot be put aside. ESBLs hydrolyse the activity of broadspectrum antibiotics thereby causing treatment failures.

In this study, the overall proportion of MBL production was at 7 (19.4 %) with *E. coli* 5 (20.8 %) and *K. pneumoniae* 2 (16.7 %). The worldwide occurrence of carbapenem resistance among the enterobacteriaceae particularly *E. coli* and *K. pneumoniae* which are the common bacteria implicated in UTIs is a worrisome situation that needs continuous surveillance [15, 25]. The overall MBL production of 19.4 % found in this study is higher than 15.26 % reported by Kulkarni and Mulay [49] against clinical isolates of *Klebsiella pneumoniae* and *E. coli*. In contrast to the present study, Fazlul et al. (52.6 %) [50] and Marie et al. (53 %) [51] found a much higher prevalence of MBL in their studies. Similarly, Kulkarni and Mulay [50] found a high MBL-positive prevalence of 43.66 % and 40.67% against *Klebsiella pneumoniae* and *E. coli* isolates respectively. The prevalence of 20.8 % and 16.7 % reported against *E. coli* and *K. pneumoniae* in our study is lower than the 38 % and 19 % reported by Shrestha et al. [52] in Nepal respectively. Studies carried out in Nigeria on the prevalence of carbapenem resistance due to MBL production were reported across all six geopolitical zones by Tula et al. [15]. They ranged from 6.8 % to 12.4 % in North-East Nigeria, 4.5 % to 77.0 %

in North-West Nigeria, 2.4 % to 100 % in North Central Nigeria, 28.0 % to 53.3 % in South-East Nigeria, 2.2 % to 93.8 % in South-West Nigeria, and 4.1 5 to 70.0 % in South-South Nigeria respectively. Increased resistance of the isolates to Beta-lactamase inhibitor and the emergence of resistance to meropenem (16.7 %) seen as the drug of last resort in the treatment of ESBL positive bacteria is of serious concerns, which underscores the need to incorporate routine ESBL and MBL detection in the management of urinary tract infections.

5. CONCLUSION

Our study recorded an overall prevalence of 17.5 % among the isolates. The entire isolates were resistant to commonly prescribed antibiotics. ESBL and MBL production was seen among the isolates. Therefore, routine screening for bacteriuria in all pregnant women and incorporation of routine ESBL and MBL detection will eliminate possible complication due to UTI in pregnancy.

CONSENT

This study was carried out with consent from the Institutional Research Committee of Akanu Ibiam Federal Polytechnic and Mater Misericordia Hospital, Afikpo, Ebonyi State, Nigeria.

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