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Prevalence and characterization of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from poultry in Ouagadougou, Burkina Faso

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This study aimed to determine the prevalence of extended spectrum beta-lactamase producing (ESBL) and multidrug resistant *Escherichia coli and Klebsiella pneumoniae.* A cross-sectional study was conducted in Ouagadougou in two poultry farms and two slaughterhouses. 375 cloacal swabs and 46 environment samples were collected and members of Enterobacteriaceae were isolated on EMB agar containing 4 µg/L of cefotaxime. *E. coli* and *K. pneumoniae* were identified using biochemical tests and ESBL production was confirmed by the double-disc synergy test. Antibiotic susceptibility was determined by the disc diffusion method. Prevalence of faecal ESBL producing *E. coli* and *K. pneumoniae* was 12.11% (95% CI = 9.3-15.6). In sampling sites, the prevalence were 5.15% in Farm A, 2.22% in Farm B, 17.50% in slaughterhouse C, 20.59% in slaughterhouse D and 19.57% in environment. *E. coli* (n = 43) and *K. pneumoniae* (n = 13) were frequently identified. ESBL-producing *E. coli* and *K. pneumoniae* MDR was 89.29% (95% CI = 78.5–95.0). Resistance to aminoglycosides was 6.25% in poultry and 10.00% in slaughterhouse, fluoroquinolones 32.5% in slaughterhouse, sulfonamides 100% in poultry and 82.50% slaughterhouse, tetracycline 100% in poultry and 95.0% in slaughterhouse. This study showed that antimicrobial resistance in poultry in Ouagadougou portends a serious problem.

Key words: *Escherichia coli*, *Klebsiella pneumoniae,* extended-spectrum β-lactamase (ESBL), poultry, farms, slaughterhouses, Ouagadougou.

INTRODUCTION

The discovery of antibiotics has been a major breakthrough in human history. However, the emergence of multidrug resistance (MDR) among pathogenic bacteria such as *Escherichia coli* and *Klebsiella*

pneumoniae is an important public health problem in human medicine, animal husbandry, veterinary medicine, and livestock management (Montso et al., 2019). These two species are associated with a wide range of infections such as pneumonia, urinary tract infections, septicemia, and soft tissue infections in humans (Ghosh et al., 2019). They also cause infections in cats, dogs, birds, horses, monkeys, pigs, rats, elephants, and poultry (Mobasseri et al., 2019; Russo et al., 2021). Specifically *E. coli* infections in chicken viz airsacculitis, cellulitis, pericarditis, perihepatitis, and respiratory distress, are critical production-limiting disease for the poultry industry (Ghosh et al., 2019).

By 2050, predictions estimate that over 10 million of deaths and ≈ nearly 100 trillion USD economic loss would result from antibiotic resistance worldwide (Maestre-Carballa et al., 2019). Although this multi-resistance to bacteria is due to the use of antimicrobials on a given chicken farm varies, used by farmers who may have less knowledge of antimicrobials, poorer bio-security practices (including housing other species in close proximity to chickens), and, consequently, a higher burden of disease is even less predictable. Globally, 63,000 tons of antibiotics are being used in livestock, which will further increase to 10,5000 tons in 2030 (Boeckel et al., 2015). These practices contribute to the widespread increase of antimicrobial resistant pathogens in human, livestock, and the environment, which consequently leads to the prolonged hospital stay for patients, financial burden to the society, and even fatal consequences (Klein et al., 2018).

Infections caused by multidrug resistant bacteria are associated with higher mortality, morbidity and healthcare costs (Ndir et al., 2016). Enterobacteriacae producing extended-spectrum-β-lactamases (ESBLs) represent main challenges to antibiotic therapy, with increasing prevalence rates throughout the world (Doi et al., 2017). In Burkina Faso, the number of clinical infections with ESBL-producing organisms is increasingly high (Ouedraogo et al., 2016). Precise information about the spreading of ESBL-organisms influenced by poultry and foodborne is poorly known; however, the proportions of ESBLs carriage in animal vary between countries and farms, but they generally range from 10 to 50% for the faecal carriage in healthy animals, and up to 95% in chicken raw meat (Saidani et al., 2019). Current knowledge concerning the presence of ESBL-producing *E. coli* and *K. pneumoniae* on poultry in Burkina Faso is limited because few studies have been carried out in this aspect. The aim of this study was to determine prevalence and characterize extended-spectrum β-lactamase-producing *E. coli* and *K. pneumoniae* isolated from poultry in Ouagadougou.

MATERIALS AND METHODS

Type, period and sampling area of study

This was a cross-sectional study conducted during

the period August 1 to November 30, 2020. Two poultry farms located in rural area of Ouagadougou designated A and B, their proximity environment (chickens drinking water, food and farms space) and two poultry slaughterhouses designated C and D were used in this study. It was:

- Farm A, located in Pabré GPS (Longitude: -1.592197, Latitude: 12.536435) which grows chickens like Isa-brown, Holland blue and Harco.
- Farm B, located in Pabré GPS (Longitude: -1.557643, latitude: 12.518307) which grows local chickens, guinea fowl, coquelet, turkey, Isa-brown.
- Slaughterhouse C, located in Kamboinsin GPS (Longitude: -1.548692, Latitude: 12.439923), which sells chickens like Isa-brown and Holland blue.
- Slaughterhouse D , located in Tanghin GPS (Longitude: -1.5158699, Latitude: 12.3946333) which sells local chickens and guinea fowl.
- Farms environment which group poultry drinking water, food, and their caecal.

Samples and data collection

Written informed consent was obtained from the poultry farms managers before enrollment. Each manager was interviewed using a questionnaire to obtain information on age, sex, poultry breed and use of antibiotics in treatment or supplementation of their poultry. Poultry were selected at random from each farm; sampled poultry were separated from others; and in slaughterhouses, 10 chickens were selected each collection day for 19 days. Cloacal samples were taken by swabbing the cloacae of each poultry, using sterile swab soaked in sterile physiological saline. Environmental samples were taken from chicken's drinking water, food, farms space. All swabs and environment samples were transported in cooler boxes and stored at 4°C.

Isolation and identification of enterobacteriaceae

On August to November 2020, a total of 375 cloacal swabs and 46 environment samples were screened. Swabs were seeded broth-heart-brain (HiMedia, india) and incubated overnight at 37˚C to improve the bacteriological yield. After this enrichment, 10 µl of the broth was transferred to eosine methylen blue (EMB) agar (HiMedia, india) supplemented with 4 µg/L of cefotaxime and incubated at 37°C for 24 h to screen for ESBL/AmpC-producing Enterobacteriaceae. Predominant colonies of different morphotypes were identified at species level using phenotypic characteristics, Gram staining, oxidase, and fermentation tests. Bacteria Gram-negative, oxidase negative and fermentation-positive isolates were biochemically identified using in-house biochemical tests (Triple sugar iron agar, Sulfur-indole-motility test, Simmons´s citrate agar, and urease test). Finally, bacterial isolates were stored at -80°C in brain heart infusion broth supplemented with 20% glycerol

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Table 1. Distribution of poultry according to age group and gender.

Characteristics		Farms		Slaughterhouse		Total
		A	B	C	D	
Sex	Cocks (n)	35	16	100	12	163
	Hens (n)	62	74	20	56	212
Age group (Month)	1-2	0	0	0	0	0
	2-3	19	61	20	12	112
	3-4	31	10	100	50	191
	4-5	0	13	0	6	19
	5-6	6	6	0	0	12
	≥6	41	0	0	0	41

n: number.

Phenotypic ESBL/AmpC testing

All *E. coli* and *K. pneumoniae* isolates were screened for ESBL production by the double-disk synergy test (DDST) using cefotaxime 30 µg, ceftazidime 30 µg , ceftriaxone 30 µg, cefepime 30 µg and amoxicillin/clavulanic 20/10 µg (HiMedia Laboratories Pvt. Ltd). Phenotypic detection of AmpC production was carried out for *E. coli* and *K. pneumoniae* strains that were either resistant to cefoxitin and/or resistant to ≥3 β-lactam antibiotics (CA-SFM, 2013).

Antimicrobial susceptibility testing

ESBL-producing *E. coli* and *K. pneumoniae* isolates were analysed by Kirby-Bauer disk diffusion technique to determine the resistance patterns of the isolates (CA-SFM, 2013). The strains were tested for antimicrobial susceptibility using the cefotaxime (CTX 30 µg), ceftazidime (CAZ 30 µg), ceftriaxone (CRO 30 µg), Cefepime (CPM 30 µg), cefoxitine (CX 30 µg), amoxicillin/clavulanic (AMC (20/10 µg), imipinem (IPM 10 µg), gentamicin (CN 10 µg), ciprofloxacin (CIP 5 µg), trimethoprim/sulfamethoxazole (SXT,1.25/23.75 µg), and Tetracycline (TE 30 µg) (HiMedia Laboratories Pvt. Ltd). Zones of inhibition were measured with a precision caliper and isolates exhibiting resistance to at least three antimicrobial agents tested were considered as multidrug-resistant strains (CA-SFM, 2013). Zone diameters were compared with the EUCAST criteria to determine if isolates were resistant, intermediate, or susceptible.

Quality control

Standard protocoles have been strictly followed during laboratory analyzes. The expiration dates of culture media, reagents and other consumables have been checked and documented. A verification of the contaminants of the media already prepared was carried out by sterility test. A quality control was carried out in order to test fertility of media. Each new batch was checked before use by testing *E. coli* ATCC 25922 which is one of the standard control strains. During the detection of ESBL *E. coli and K. pneumoniae*, *K. pneumoniae* ATCC 700603 (ESBL positive) and *E. coli* ATCC 25922 were used as controls for this study.

Statistical analysis

Data entry was performed and statistical analysis of the results was done using XLSTAT 2017 version 19.5. The distributions of the variables were compared by the χ^2 independence test. The significance level was set at 5%.

RESULTS

Characteristics of poultry

Distribution of poultry according to age group and gender

The majority of the birds included were hens with a frequency of 212/375 (56.53% CI= 51.5-61.5). The overall sex ratio was 0.76. The minimum age was 2 months with a maximum of 20 months (Table 1).

Distribution of poultry according to breed and notion of antibiotics supplementation

According to the breed of poultry, the Isa-brown breed was the majority (107/187) (57.21% CI=50.1 -64.1) followed by Holland blue (66/187) (35.29% CI=28.8-42.4). Oxytetracycline and polymixin were used in all breeds on both intensive livestock farms (Table 2).

Prevalence of *Escherichia coli* and *Klebsiella pneumoniae*

The 421 samples analysed originated mostly from poultry farms (n=187), slaughterhouse (n=188) and poultry environment (n=46). *E. coli* and *K. pneumoniae* isolates were detected in 51 out of 421 samples analyzed (12.11%) (95% CI = 9.3-15.6). *E. coli* and *K. pneumoniae* were isolated from all four sites (farms and slaughterhouse) but with varying proportions: 5/97 (5.15%) in Farm A, 2/90 (2.22%) in Farm B, 21/120 (17.50%) in Slaughterhouse C, 14/68 (20.59%) in

Table 2. Distribution of poultry according to breed and antibiotics used.

Characteristics		Poultry breed				Total (n)
		Isa-brown (n)	Harco (n)	Holland blue (n)	Turkey (n)	
Farms	A	47	8	42	0	97
	B	60	0	24	6	90
Antibiotics	Oxytetracycline	107	8	66	6	187
	Colistine	107	8	66	6	187
	Quinolones	47	8	42	0	97
	Phenicol	60	0	24	6	90
	Amoxicilline	47	8	42	0	97

n: number.

Table 3. Frequency of ESBL producing *E. coli* and *K. pneumoniae* isolated from farms and slaughterhouse according to sampling area.

	Code	No.	Positive	Prevalence (%)	95% CI
Farms	A	97	5	5.15	2.2 - 11.5
	B	90	2	2.22	0.6 - 7.8
Slaughterhouse	C	120	21	17.50	11.7 - 25.3
	D	68	14	20.59	12.7 - 31.7
Environment	E	46	9	19.57	10.6 - 33.2
Total		421	51	12.11	9.3 - 15.6

No.: Number, CI: Confidence internal.

Slaughterhouse D, and 9/46 (19.57%) in Environment farm (Table 3). *E. coli* (n = 43) and *K. pneumoniae* (n = 13) were frequently identified bacterial species.

Prevalence of conjugative ESBL-AMPc-producing *E. coli and K. pneumoniae*

A total of 421 from poultry and slaughterhouse were further analysed. Combined ESBL- and AmpC-producing phenotypes were observed in 4 of 421 (0.95%) of the isolates.

Distribution of multi-drugs resistance *E. coli* and *K. pneumoniae* among study area

ESBL-producing *E. coli* and *K. pneumoniae* resistant to more than three antimicrobial classes, was 50/56 (89.29%) (95% CI = 78.5–95.0). Resistance to the aminoglycoside, fluroquinolonones, sulfonamide, and tetracycline classes were dominant, observed in 8.93, 23.21, 87.5 and 96.43% of the isolates, respectively. Among site of sampling, frequency of MDR were high to Slaughterhouse C (42.00% CI=29.4-55.8), Slaughterhouse D (26.00% CI= 15.9-39.6 and Farms environment (18.00%, 95% CI = 9.8-30.8) (Figure 1).

Antimicrobial resistance of all *Escherichia coli* and *Klebsiella pneumoniae* strains

All isolates were sensitive to carbapenems. Prevalence was observed in ciprofoxacin (32.5% in slaughterhouse), gentamicin (6.25% in poultry and 10.00% in slaughterhouse), sulfamethoxazole – trimethoprime (100% in poultry and 82.50% slaughterhouse), tetracycline (100% in poultry and 95.0% in slaughterhouse), cefoxitin (2.5% in slaughterhouse), and amoxicillin/clavulanic acid (100% in poultry and 95.0% in slaughterhouse). Absolute resistance was observed in cefotaxime (100%), ceftriaxone (100%), cefepime (100%) and ceftazidime (100%) (Figure 2).

DISCUSSION

During the study, prevalence (12.11%, 95% CI = 9.3-

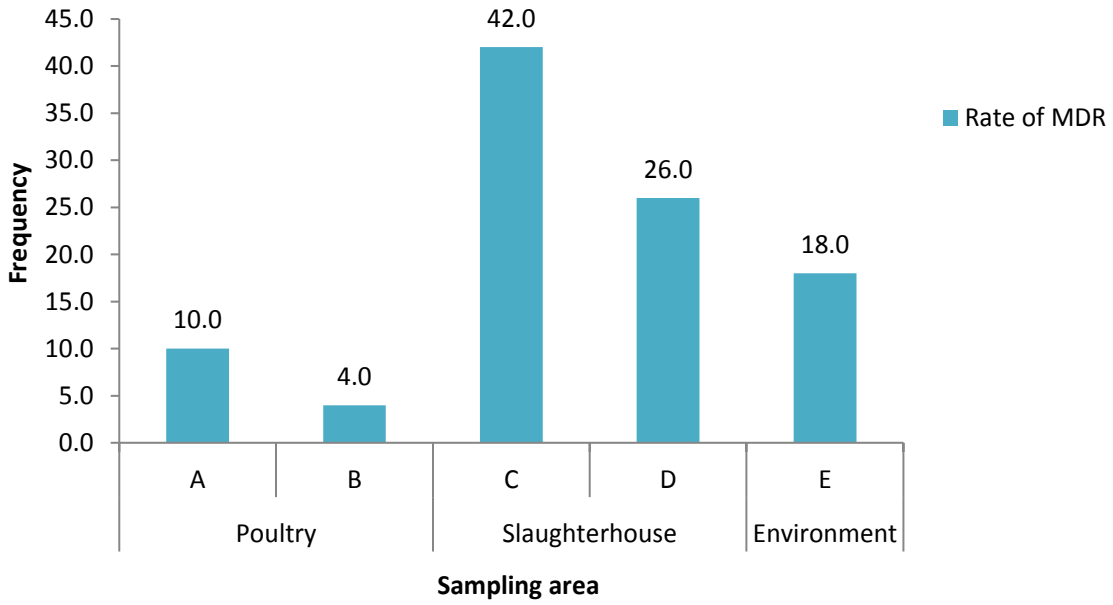

Figure 1. Repartition of MDR isolates from poultry farms, slaughterhouses and farms environment in Ouagadougou.

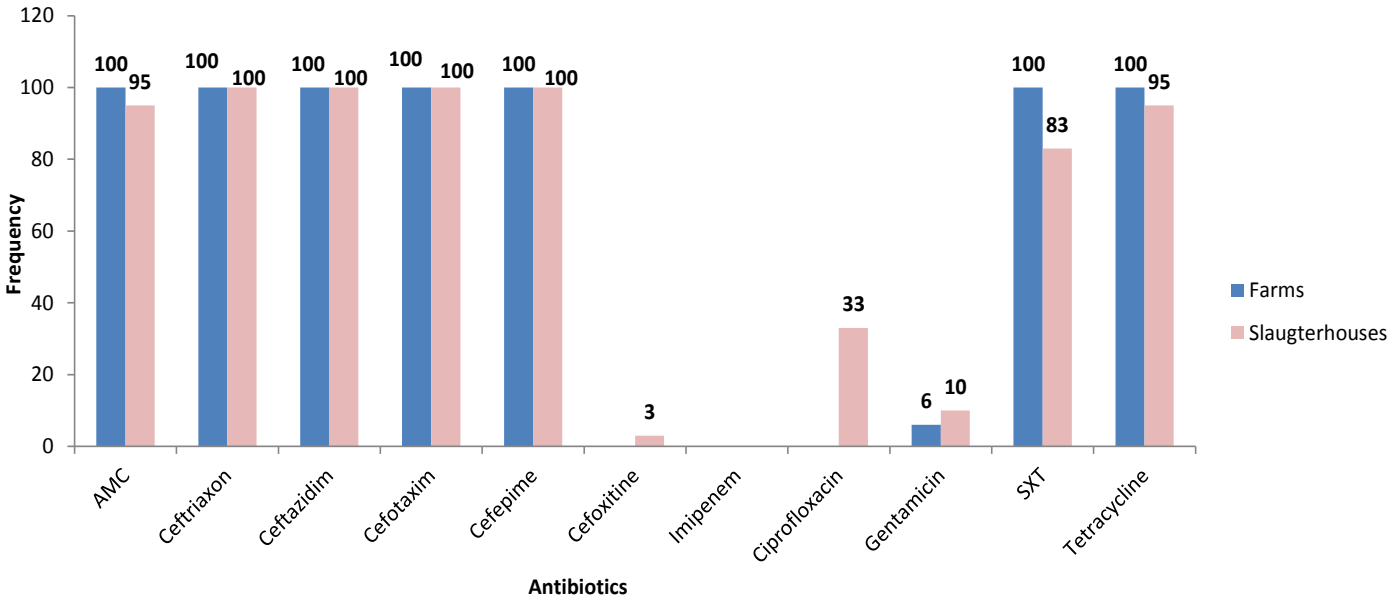

Figure 2. Percentage of antimicrobial resistance of all *Escherichia coli* and *Klebsiella pneumoniae* strains in this study.
AMC: Amoxiccilline/clavulanic acid, SXT: sulfamethoxazole – trimethoprime.

15.6) of ESBL producers was seen in isolates from poultry farms, slaugterhouse and farms environment. This frequency could be explained by the fact that antibiotics had been widely used in healthy animals for growth promotion, a practice now banned in West Africa and in other countries, but still active in others (Maamar et al., 2016). In Burkina Faso, the use of antibiotics is excessive and not controlled in animal health (Samandoulougou et al., 2015). This high prevalence of ESBL producing *E. coli* and *K. pneumoniae* detected in poultry feces could increase human fecal carriage if the microbial flora of the chicken meat is not well inactivated. This frequency of multiresistant enterobacteria isolated in poultry could be concealed in humans and environment by the consumption of contaminated meat. The lack of bio-security in some farms could explain the possible

dissemination of these clonal isolates. ESBL/AmpC producing strains might have been transmitted vertically from breeding flocks to chicks and established in the poultry environment (Vounba et al., 2019). Under the pressure of antibiotic selectivity, drug-resistant bacteria emerged, disseminated in healthy poultry and can spread to humans through consumption of contaminated food, from direct contact with poultry, or by environmental spread (Montso et al., 2019). In the present study, in all two farms, two poultry slaughterhouses and environment poultry were colonized by ESBL producers *E. coli* isolates. The rate of fecal carriage of these multidrug resistant bacteria in healthy chickens varied from one site to another (2.22 to 20.59%). These results confirm that chicken farms constitute a reservoir of ESBL producing *E. coli* and *K. pneumoniae* isolates, which might reflect a high antibiotic pressure for selection of resistant bacteria in this ecosystem (Umair et al., 2019; Saidani et al., 2019; Ghosh et al., 2019; Mobasseri et al., 2019). This prevalence is higher than those observed in Bobo Dioulasso in 2019 where the poultry faecal carriage of ESBL producing *E .coli* and *K. pneumoniae* was 0.8% (Sanou et al., 2019). However, our results are similar to those found in Pakistan where prevalence rate was 13.7% (Umair et al., 2019) and Tunisie where prevalence was between 4 and 67.3% (Saidani et al., 2019). In our study, like in other several countries, cephalosporins are not used for poultry, but high prevalence of ESBL-producing bacteria remains (Sanou et al., 2019). This suggests that there are additional sources for the contamination with ESBL-producing bacteria in livestock. Our situation may be explained by an environmental contamination. Indeed, ESBL-producing bacteria may spread to environment by waste products from human activities and animal production (Soré et al., 2020).

The ESBL *E. coli* and *K. pneumoniae* showed any resistance to imipenem. These two species also showed resistance to ciprofoxacin (33.0% in slaugtherhouse and 0% in farms), gentamicin (10.0% in slaugtherhouse and 6.0% in farms) and sulfamethoxazole – trimethoprime (83.0% in slaugtherhouse and 100% in farms), tetracycline (95.0% in slaugtherhouse and 100% in farms). This resistance to fluoroquinolones, aminoglycosids, sulfamethoxazole – trimethoprime and tetracyclines might have resulted from overuse due to easy access, and lack of control of these antibiotics in the market. In addition to that, most isolates presented multiple-associated resistances, highlighting that ESBL producing *E. coli* can be selected using other veterinary-licensed antibiotics besides broad-spectrum cephalosporins (Diab et al., 2017). Plasmids carrying genes encoding ESBLs is known to also carry other genes conferring resistance to fluoroquinolones, aminoglycosides, tetracycline, and cotrimoxazole (Ouédraogo et al., 2017). The high resistance of cephalosporins explained by Ceftiofur was still systematically used at the hatchery to prevent omphalitis in broiler chicken farms during the period of our sample collection, which could therefore explain the high prevalence of cephalosporin resistance, as demonstrated by a previous study from France (Vounba et al., 2019). However, gentamicin resistance was not highly prevalent although it is not used as aminoglycosides in poultry in the country (Samandoulougou et al., 2015).

Of the 56 ESBL *E. coli* and *K. pneumoniae*, 50 isolates (89.29%) showed multidrug resistance that is resistance to at least three antimicrobials (Figure 1). This prevalence of MDR Enterobacteriacae, was 14.0% in farms, 68.0% slaughterhouses and 18.0% in farms environment. All the strains from farms and farm environment showed resistance to tetracycline, which is used widely in feed supplements (Mobasseri et al., 2019). In general, *E. coli* and *K. pneumoniae* MDR are becoming a serious issue in humans and animals, with an increasing resistance to most available antibiotics (Mobasseri et al., 2019; Stuart, 2002). The presence of these various plasmids, often mediating resistance to several antimicrobials, could also explain the high prevalence of MDR mentioned previously (Vounba et al., 2019).

Conclusion

The results shown in the present investigation emphasize the role of poultry in the spread of ESBL-producing *E coli* and *K. pneumoniae*, and the risk that these microorganisms can reach humans through the food chain. Therefore, close surveillance of antimicrobial resistant bacteria from poultry and food production livestocks should be established as a priority. Almost all of the isolates were MDR, with resistance to major antibiotics used in human medicine such as broad spectrum beta-lactams, fluoroquinolones, aminoglycosides, trimethoprim– sulfamethoxazole and tetracycline. However, in order to avoid the selection of resistant mutants, farmers should refrain from supplementing antibiotics in poultry feed. Also, in order to reduce the dissemination of these multiresistant bacteria in the environment and in humans, a strengthening of hygiene at the level of farms and slaughterhouses is recommended.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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