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Research Article

Susceptibility of extended-spectrum ß-lactamase (ESBL)-producing *Enterobacteriaceae* to Roundup

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Abstract

Bacteria and other microorganisms have several mechanisms to react to stress in the environment. Exposure of bacteria to antibiotics, biocides, or selective pressure may favor the emergence of antimicrobial resistance by several mechanisms as an evolution principle. Bacteria may possess cross-tolerance or cross-resistance to other environmental toxic substances present in soil, water, foods, and feeds. Glyphosate (N-phosphonomethylglycine), one of these substances used in modern agriculture might change the susceptibility of bacteria to antibiotics. The present study aimed to investigate the tolerance of extended-spectrum ß-lactamases (ESBL)-producing *Enterobacteriaceae* isolated from patients with nosocomial infections to glyphosate. Therefore, the minimum inhibitory concentrations of glyphosate-based herbicide (Roundup) of ESBL-positive and ESBL-negative *Enterobacteriaceae* were determined. Results showed that ESBL-producing *Enterobacteriaceae* exhibited a higher tolerance to Roundup compared with non-ESBL. To investigate the putative link between ESBL-producing *Enterobacteriaceae* and the resistance to glyphosate, a non-ESBL *E. coil* strain was used for development of glyphosate-resistant mutants using high concentrations of Roundup. Nine Roundup-resistant mutants were developed and characterized using Matrix-Assisted Laser Desorption/Ionization-Time of Flight. One Roundup-resistant mutant (Mut-A) different antibiotic susceptibility profiles compared with wild type strain. The Mut-A developed resistance to ampicillin/sulbactam, piperacillin, and streptomycin. Overall, herbicides resistant *Enterobacteriaceae* might render resistant to ß-lactam antibiotics as well. Further studies are urgently needed to investigate the mechanism of the putative link between antibiotic resistance and the herbicide-based glyphosate.

Keywords: Glyphosate, Roundup, Antimicrobial Resistance, Mutation, Antibiotics

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Introduction

To survive in the environment, microorganisms can develop or gain several mechanisms to react quickly to stressful situations that may arise. As a response to lack of nutrients or presence of toxic substances such as antimicrobial compounds, bacteria modify their metabolism to survive. For example, levels of nicotinamide adenine dinucleotide and adenosine triphosphate act as signaling molecules in both Gram-positive and Gram-negative bacteria to activate stress factors and control oxidative stress (Proctor and von Humboldt, 1998). Additionally, selective pressure due to exposure to antibiotics or biocides may lead the bacteria to develop resistance patterns (Russell, 2003), causing a major existential threat. Besides antibiotic and biocide, bacteria may develop resistance to other environmental toxic substances in soil, water, foods, and feeds.

Glyphosate (N-phosphonomethylglycine), a widely used herbicide in modern agriculture, has recently attracted attention due to its antimicrobial activity. It inhibits the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme, which plays a role in the shikimic acid pathway, namely biosynthesis of aromatic amino acids and other secondary metabolites including vitamin K, tetrahydrofolate, and ubiquinone in plant cells (Gruys and Sikorski, 1998). The EPSPS enzyme converts phosphoenolpyruvic acid (PEP) and 3-phosphoshikimic acid (S3P) into 5-enolpyruvyl-3-phosphoshikimic acid. Inhibition of this enzyme leads to shutting down of the shikimate pathway which subsequently inhibits the biosynthesis of aromatic amino acids resulting in death of plant cells (Jones, 1999; Cerdeira and Duke, 2006).

The extensive use of glyphosate as an herbicide in crop production can lead to residues of the active substance and related metabolites in the food chain. Glyphosate residues were detected in the en-

vironment (Erickson et al., 2003; Bekker et al., 2014; Noori et al., 2018), cattle (Krüger et al., 2014), pets (Knapp et al., 2013), chickens (Shehata et al., 2014), soybeans (Duke et al., 2018; Stephenson et al., 2018), feed (Reuter et al., 2007; Reddy et al., 2018; Zhao et al., 2018), and human samples (Zouaoui et al., 2013; Gillezeau et al., 2019). Glyphosate exhibited also antibacterial effects (Shehata et al., 2013a), and it was patented as a broad-spectrum antimicrobial (William, 2002). Some pathogenic isolates are resistant to glyphosate compared to commensal microflora that might lead to dysbiosis (Shehata et al., 2013a). Moreover, new *Salmonella* isolates exhibited more resistance to glyphosate more than *Salmonella* that isolated before broad usage of glyphosate (Pöppe et al., 2019). However, in cattle model, it was found that glyphosate has no relevant effect on intestinal microbiota (Billenkamp et al., 2021). The authors explained this effect due to possible adaptation of microbiota to glyphosate exposure.

Sub-lethal concentrations of glyphosate could influence the antibiotic susceptibility (Kawamura et al., 2017; Abayneh and Worku, 2020; Denkel et al., 2020). Kurenbach and others found that transient exposure to sub-inhibitory concentrations of glyphosate alters antibiotic susceptibility profiles (Kurenbach et al., 2015), however, Pöppe and co-workers found that *Salmonella enterica* mutants induced experimentally by glyphosate do not increase the cross tolerance or cross resistance to antibiotics (Pöppe et al., 2020).

Considering the fact that the main proportion (61%) of glyphosate is excreted in feces (von Soosten et al., 2016), the role of these residues might play a role in the emergence of extended-spectrum ß-lactamases (ESBL)-producing *Enterobacteriaceae*, attracted a lot of attention recent years (Abayneh and Worku, 2020; Denkel et al., 2020). Therefore, in the current study we investigated the susceptibility of nosocomial pathogens including *E. coli*, *Klebsiella (K.) pneumoniae*, *Enterobacter (E.) cloacae* and *Proteus (P.) mirabilis* to ß-lactam and glyphosate. Additionally, the putative link between ß-lactam resistance conveyed by extended ß-lactamases in *Enterobacteriaceae* and glyphosate was investigated.

Material and Methods

Bacterial isolates

Forty-five ESBL producing isolates (26 *E. coli*, 14 *K. pneumoniae*, 3 *E.cloacae*, 2 *P. mirabilis* and twenty-seven non-ESBL producing isolates (16 *E. coli*, 5 *K. pneumoniae*, 3 *E. cloacae*, 3 *P. mirabilis*) isolated at the Institute of Medical Microbiology and Epidemiology of the University Hospital of Leipzig from different sources. More details are shown in Table 1.

Investigations of antibiotic resistance

The minimum inhibitory concentration (MIC) values for a panel of 22 antibiotics were determined by broth microdilution technique according to DIN EN ISO 20776-1: 2006. The ESBL producing isolates were confirmed using the Etest® (bioMérieux) conducted in triplicate according to the manufacturer's instructions. The tested antibiotics are listed in Table 2.

Investigations of glyphosate resistance

The MIC value of Roundup was determined using Roundup UltraMax® in triplicate in 24-well microtiter plates. Briefly, 100 µl of bacterial suspension (10^5 CFU/mL) were added to 900 µL reinforced clostridial medium (RCM, Sifin, Berlin, Germany) containing different concentrations of Roundup (5.0, 2.4, 1.2, 0.6, 0.3, 0.15 and 0.075 mg/mL) and then incubated at 37°C. Results were read after 24 h of incubation. Bacterial growth was evaluated on nutrient agar (Sifin, Berlin, Germany) and the MIC value was determined as the lowest concentration inhibiting the growth of the tested bacteria.

Selection of glyphosate mutants from antibiotic susceptible *E. coli*

The antibiotic sensitive *E. coli* (B253) strain isolated from patients suffering from renal infections and proved to be vulnerable to the tested antibiotics was used. The Roundup-resistant mutants were generated as previously described with some modifications (Shehata et al., 2013a). Briefly, 100 µL of the *E. coli* (B253) suspension (10^5 CFU/mL) were added to 900 µL of RCM supplemented with 400 µg/mL Roundup and incubated for 24h at 37°C. The suspension was subcultured on blood agar (Sifin, Berlin, Germany) containing 2.4 mg/mL Roundup and incubated for further 48-72 h at 37°C. Colonies were passaged five times on blood agar containing 2.4 mg/mL Roundup. Stable diminished colony sizes were determined after 30 passages on blood agar, and these criteria served as a principle of stability (Shehata et al., 2020). Nine Roundup-resistant clones were stable and analyzed using Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) (Shehata et al., 2013a). These clones were used for further studies to investigate the influence of glyphosate resistance on antibiotic susceptibility as outlined above.

Results

Antimicrobial and Roundup resistance pattern of *E. coli*

All ESBL-positive *E. coli* strains (N=26) exhibited resistant to cefotaxim, cefuroxim, ampicillin, aztronam, ceftazidime, and piperacillin, were susceptible to imipenem, meropenem, colistin, and doripenem. While the rates of susceptibility of ESBL-positive *E. coli* strains to amikacin, ampicillin/sulbactam, ciprofloxacin, gentamycin, moxifloxacin, piperacillin, cotrmoxzol, ceftibuten, ertapenem, fosfomycin, levofloxacin and tobramycin were 88%, 12%, 38%, 73%, 38%, 77%, 19%, 23%, 92%, 85%, 31% and 69%, respectively. On the other hand, all ESBL-negative *E. coli* strains (N=11) were resistant only to cefuroxime. The rates of susceptibility of ESBL-negative *E. coli* strains to ampicillin/sulbactam, cefotaxim, gentamycin, cotrmoxzol, ampicillin, fosfomycin and piperacillin were 56%, 94%, 88%, 63%, 38%, 75% and 50%, respectively. Interestingly, ESBL-positive, and

Table 1: ESBL and non-ESBL *Enterobacteriaceae* used in this study

Species	ESBL-positive	ESBL-negative	Origin
E. coli	13	10	Urine
E. coli	7	1	Wound swab
E. coli	2	-	Skin swab
E. coli	4	5	Other locations
K. pneumoniae	10	3	Urine
K. pneumoniae	4	2	Other locations
P. mirabilis	1	1	Other locations
P. mirabilis	1	2	Urine
E. cloacae	2	1	Urine
E. cloacae	1	2	Other locations

Table 2: Antibiotic and Roundup resistance of ESBL-positive and -negative *E. coli* strains

Antibiotic	ESBL-positive (N= 26)		ESBL-negative (N= 11)	
	Range (mg/L)	Susceptibility %	Range (mg/L)	Susceptibility %
Amikacin	1-16	88	1-8	100
Ampicillin/Sulbactam	4- >32	12	0.5->32	56
Ciprofloxacin	⩽0.031-4	38	⩽0.031-0.063	100
Cefotaxim	>8	0	⩽0.063-0.125	94
Cefuroxim	>32	0	2-8	0
Gentamycin	0.25-512	73	⩽0.125-1	88
Imipenem	⩽0.125-0.5	100	⩽0.125-0.5	100
Meropenem	⩽0.125	100	⩽0.125	100
Moxifloxacin	<0.031->4	38	⩽0.031-⩽0.063	100
Pieracillin	0.5-64	77	1-4	100
Cotrmoxzol	⩽0.125->16	19	⩽0.125->16	63
Ampicillin	>32	0	2->32	38
Aztronam	4->16	0	⩽0.25-0.5	100
Ceftazidim	2—32	0	⩽0.25-0.5	100
Ceftibuten	0.25-4	23	0.125-1	100
Colistin	0.25-1	100	0.25-1	100
Ertapenem	0.031-4	92	⩽0.031	100
Doripenem	0.125-0.5	100	⩽0.125-0.25	100
Fosfomycin	⩽1-64	85	4-32	75
Levofloxacin	⩽0.063->8	31	⩽0.063	100
Piperacillin	>64	0	1.0>64	50
Tobramycin	0.25-16	69	0.25-0.5	100
Roundup (mg/mL)	0.6-2.4	22	0.3-0.6	100

ESBL-negative *E. coli* strains exhibited different susceptibility to glyphosate. A total of 78% of the ESBL-positive *E. coli* strains had a MIC value of 2.4 mg/mL Roundup, while all ESBL-negative *E. coli* strains had MIC values of 0.3 or 0.6 mg/mL Roundup (Table 2 and Figure 1).

Antimicrobial and Roundup resistance pattern of *K. pneumoniae*

The ESBL-positive *K. pneumoniae* strains (N=14) were resistant to ampicillin/sulbactam, ciprofloxacin, cefuroxim, piperacillin, ampicillin, ceftazidim, fosfomycin, and piperacillin. The rates of susceptibility of ESBL-positive *K. pneumoniae* strains to amikacin, cefotaxim, gentamycin, meropenem, moxifloxacin, cotrmoxzol, aztronam, ceftibuten, colistin, ertapenem, doripenem, levofloxacin, and tobramycin were 79%, 7%, 50%, 93%, 14%, 14%, 7%, 43%, 86%, 57%, 93%, 29%, and 43%, respectively. All (14/14) ESBL-positive *K. pneumoniae* strains exhibited susceptibility to imipenem. On the other hand, all non-ESBL *K. pneumoniae* strains (N=5) were resistant to cefuroxime, ampicillin, and fosfomycin. The rate of susceptibility of non-ESBL *K. pneumoniae* strains to ampicillin/sulbactam, cotrmoxzol, colistin, and piperacillin was 80%. Ninety-three percent of the ESBL-positive *K. pneumoniae* strains had a Roundup MIC value of 2.4 mg/mL. In contrast, 100% of the ESBL-negative strains had a Roundup MIC of 0.6 mg/mL (Table 3 and Figure 1).

Antimicrobial and Roundup resistance pattern of *E. cloacae*

The ESBL-positive *E. cloacae* strains (N=3) were resistant to amikacin, ampicillin/sulbactam, cefotaxim, cefuroxim, imipenem, meropenem, colistin, doripenem, fosfomycin, levofloxacin, and piperacillin. However, all ESBL-positive *E. cloacae* strains were susceptible to ampicillin, aztronam, ceftazidim, and ceftibuten. All non-ESBL *E. cloacae* strains (3/3) were resistant to cefuroxime, ampicillin, and fosfomycin. On the other hand, all ESBL-negative *E. cloacae* (3/3) were susceptible to amikacin, ciprofloxacin, cefotaxim, gentamycin, imipenem, meropenem, moxifloxacin,

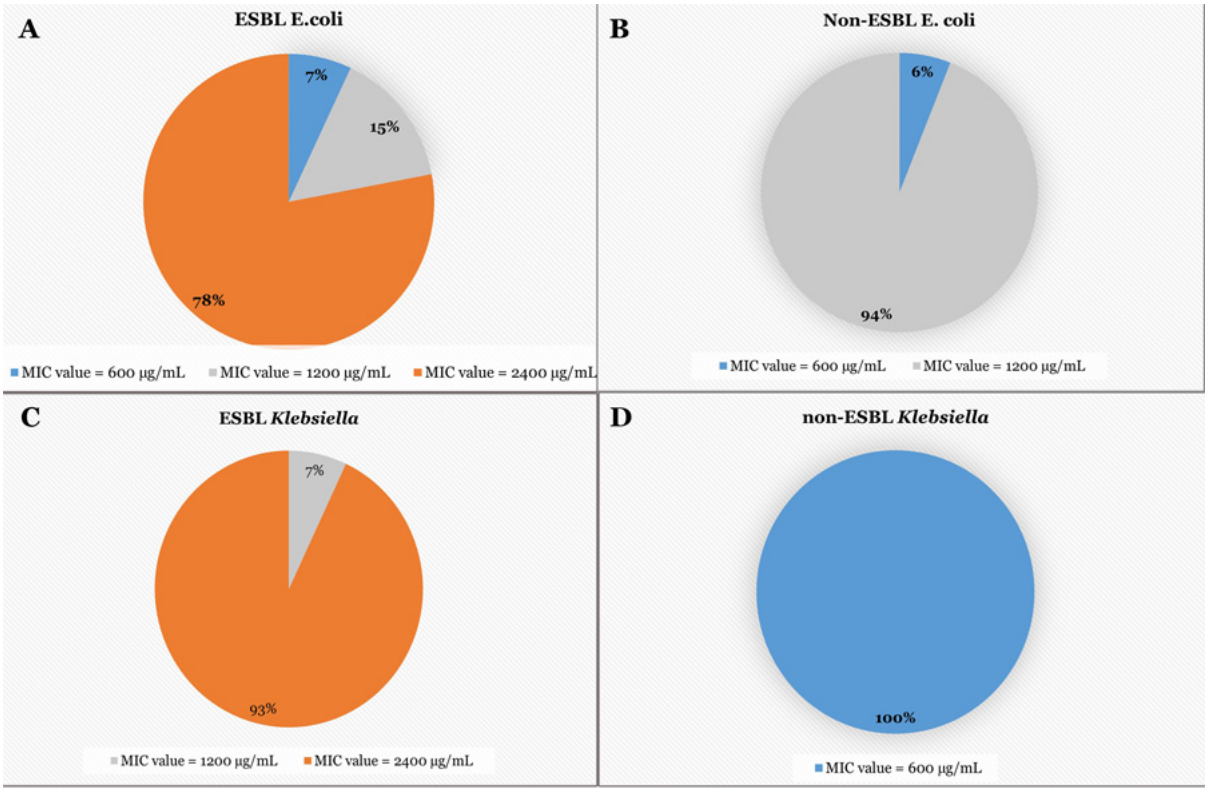

Figure 1: Minimum inhibitory concentration (MIC) of extended-spectrum ß-lactamases (ESBL) *E.coli* (A), non-ESBL *E. coli* (B), ESBL *Klebsiella* (C) and non-ESBL *Klebsiella* (D) to Roundup

piperacillin, aztronam, ceftazidim, ceftibuten, colistin, ertapenem, doripenem, levofloxacin, piperacillin, and tobramycin. Although, few *Enterobacter* isolates were investigated, the ESBL-positive *E. cloacae* showed high resistance to Roundup (MIC of 2.4-5.0 mg/mL Roundup), while the ESBL-negative *E. cloacae* strains had a MIC of 0.6-1.2 mg/mL Table 4.

Antimicrobial and Roundup resistance pattern of *P. mirabilis*

The ESBL-positive *P. mirabilis* strains (N=2) were resistant to amikacin, ampicillin/sulbactam, cefotaxim, cefuroxim, ampicillin, aztronam, ceftazidim, colistin, and piperacillin. The two strains were susceptible to meropenem, piperacillin, levofloxacin, and tobramycin. All (3/3) ESBL-negative *P. mirabilis* strains were resistant to cotrmoxzol and colistin and were susceptible to amikacin, ampicillin/sulbactam, ciprofloxacin, cefotaxim, imipenem, meropenem, moxifloxacin, piperacillin, ampicillin, aztronam, ceftazidim, ceftibuten, ertapenem, doripenem, levofloxacin, piperacillin, and tobramycin. Interestingly, both ESBL-positive and ESBL-negative *P. mirabilis* isolates were Roundup resistant, independent of their ESBL status (Table 5).

Selection of Roundup mutants from formerly antibiotic susceptible *E. coli* (B253)

Nine Roundup resistant mutants designated Mut-A, Mut-N, Mut-F, Mut-2, Mut-3, Mut-5, Mut-6, Mut-8, and Mut-32 were recovered from ESBL-negative *E. coli* B253. Mutants were confirmed as *E. coli* based on biochemical tests and MALD-TOF analysis. Out of the 9 Roundup resistant mutants, one mutant (Mut-A) developed resistance to ampicillin/sulbactam, piperacillin (Table 6). The MIC value of ampicillin were for Mut-A more than 32 mg/L, while it was 1 mg/L for *E. coli* (B253) wild type strain. However, the MIC values of piperacillin were 1 and 32 mg/L for *E. coli* (B253) wild type strain and Mut-A, respectively.

Discussion

Although the International Agency for Research on Cancer (IARC) re-evaluated the risk of glyphosate and classified it as probably carcinogenic to humans (IRAC, 2015), a debate about its safety still existing for two reasons: 1) the long-term toxicology of the sub-lethal concentrations of glyphosate was not investigated in both humans and animals in details. 2) it is proposed that glyphosate is safe for humans and animals due to the absence of EPSPS enzyme. However, the inhibition of EPSPS is not the only activity of glyphosate in warm-blooded animals. Additionally, microorganisms including microflora possess EPSPS enzyme too. Due to the antimicrobial effect of glyphosate, chronic exposure of bacteria to low concentrations may drive the de novo evolution of resistance and cross-resistance to antibiotics. In the present study, the link between ß-lactam resistance conveyed by ESBL-producing *Enterobacteriaceae* and glyphosate-based herbicide resistance was demonstrated.

Indeed, some bacteria, fungi, and protozoa possessing EPSPS enzyme are also sensitive to glyphosate

Table 3: Antibiotic and Roundup resistance of ESBL and non-ESBL *K. pneumoniae*

Antibiotic	ESBL-positive (N= 14)		ESBL-negative (N= 5)	
	Range (mg/L)	Susceptibility %	Range (mg/L)	Susceptibility %
Amikacin	⩽0.5->64	79	1.0	100
Ampicillin/Sulbactam	32->32	0	4-16	80
Ciprofloxacin	⩽0.031-> 4	0	0.031-0.063	100
Cefotaxim	0.125>-8	7	⩽0.063	100
Cefuroxim	8- >32	0	2-4	0
Gentamycin	⩽0.125-512	50	0.125-0.5	100
Imipenem	⩽0.125-1	100	⩽0.125-0.5	100
Meropenem	⩽0.125- 2	93	<0.125	100
Moxifloxacin	⩽0.063-4	14	⩽0.063-0.125	100
Pieracillin	2->64	0	2-8	100
Cotrmoxzol	0.25->16	14	0.25-16	80
Ampicillin	>32	0	32->32	0
Aztronam	0.5->16	7	⩽0.25	100
Ceftazidim	2->32	0	⩽0.25-0.5	100
Ceftibuten	0.125->4	43	0.063	100
Colistin	0.25-8	86	0.25-8	80
Ertapenem	⩽0.031-4	57	⩽0.031	100
Doripenem	⩽0.125-8	93	⩽0.125	100
Fosfomycin	>0.128	0	⩽0.125	0
Levofloxacin	⩽0.063->8	29	⩽0.063	100
Piperacillin	>64	0	4-16	80
Tobramycin	0.125-16	43	0.25	100
Roundup (mg/mL)	1.2-2.4	0	0.6	100

(Clair et al., 2012). Other bacteria may be tolerant or resistant to glyphosate as their EPSPS includes a Q-loop region with an increased polarity as a unique feature (Carr et al., 2011). Glyphosate resistance development may occur due to changes in the EPSPS active site (Rainio et al., 2021). Two classes of EPSPS that share less than 50% amino acids identity were identified in bacteria (Fitzgibbon and Braymer, 1990). Class-I EPSPS, found in plants and bacteria, is naturally sensitive to glyphosate. In contrast, class-II EPSPS has a natural tolerance to glyphosate and has a high affinity to phosphoenolpyruvate. Class-II EPSPS was identified in certain bacteria such as *Pseudomonas* spp. (Fitzgibbon and Braymer, 1990), *Agrobacterium tumefaciens*, *Clostridium perfringens*, *Clostridium acetobutylicum*, and *Fusobacterium nucleatum* (Carr et al., 2011). Additionally, *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Salmonella* Gallinarum isolated from poultry exhibited resistance to glyphosate (Shehata et al., 2013b).

We found that the ESBL-producing *Enterobacteriaceae* are also resistant to Roundup (Table 2 and Figure 1), raising questions about the putative link between glyphosate resistance and emergence of antibiotic resistance. The ESBL status of *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *E. cloacae* was correlated with the Roundup resistance. There are different methods that explain the emergence of antimicrobial resistance. The widespread use and sometimes misuse of antibiotics are the major cause of emerging of antimicrobial resistance, causing a global threat to human and animal health (Principi and Esposito, 2016; Tomson and Vlad, 2014; White and Hughes, 2019; Kim et al., 2021). Moreover, some chemicals can cause antibiotic resistance through different mechanisms. Salicylic acid for instance induces antibiotic resistance in *E. coli* by changing the influx and efflux of antibiotics (Price et al., 2000). It induces tolerance to cephalosporin antibiotics due to a reduction of the expression of OmpF in *Salmonella* Typhimurium (Choi et al., 2018). Kurenbach and colleagues found that chemicals that commonly used in agriculture, domestic gardens, and public places can induce a multiple antibiotic resistant phenotype in potential pathogens (Kurenbach et al., 2015). Additionally, sub-lethal glyphosate concentrations could change the antibiotic susceptibility profiles in different bacteria (Capita et al., 2014; Kurenbach et al., 2015, 2017).

To find a link between glyphosate resistance and emergence of antibiotic resistance glyphosate, resistant mutants were developed from ESBL-negative *E. coli*. Interestingly, one mutant (Mut-A) developed resistant to ampicillin/sulbactam, piperacillin, and streptomycin resistant mutant (Mut-A) out of nine Roundup-resistant strains from Roundup-sensitive *E. coli* B253. The role of the surfactant in the Roundup and its negative impacts should be also considered (Cherepenko and Hovorun, 2005; Kurenbach et al., 2017). Although we could not study the mechanism of the effect of Roundup in the emergence of antibiotic resistance, different pathways could explain this process. Cherepenko and Hovorun found that mutations resulting in target and ligand sequestration in glyphosate resistant *Enterobacteriaceae* may render them also resistant to ß-lactam antibiotics (Cherepenko and Hovorun, 2005). The magnitude of the glyphosate induced response may undermine antibiotic therapy and substantially increase the probability of spontaneous mutation to

Table 4: Antibiotic and Roundup resistance of ESBL-positive and –negative *E. cloacae* strains

Antibiotic	ESBL-positive (N= 3)		ESBL-negative (N= 3)	
	Range (mg/L)	Susceptibility %	Range (mg/L)	Susceptibility %
Amikacin	1-16	0	1-2	100
Ampicillin/Sulbactam	4- >32	0	4-16	67
Ciprofloxacin	⩽0.031-4	33	⩽0.031	100
Cefotaxim	8	0	⩽0.063-0.25	100
Cefuroxim	>32	0	2-4	0
Gentamycin	0.25-512	33	0.125-0.25	100
Imipenem	⩽0.125-0.5	0	0.25-1	100
Meropenem	⩽0.125	0	⩽0.125	100
Moxifloxacin	<0.031->4	67	⩽0.031	100
Pieracillin	0.5-64	33	2.0	100
Cotrmoxzol	⩽0.125->16	67	0.25-16	67
Ampicillin	>32	100	>32	0
Aztronam	4->16	100	<0.25	100
Ceftazidim	2-32	100	<0.25	100
Ceftibuten	0.25- 4	100	0.25-0.5	100
Colistin	0.25-1	0	0.25-0.5	100
Ertapenem	0.031-4	33	⩽0.031-0.125	100
Doripenem	0.125-0.5	0	⩽0.125	100
Fosfomycin	⩽1-64	0	64-128	0
Levofloxacin	⩽0.063->8	0	⩽0.063	100
Piperacillin	>64	0	2	100
Tobramycin	0.25-16	33	0.25	100
Roundup (mg/mL)	2.4-5	0	0.6-1.2	33

Table 5: Antibiotic and Roundup resistance of ESBL-positive and -negative *P.* mirabilis strains

Antibiotic	ESBL-positive (N= 2)		ESBL-negative (N= 3)	
	Range (mg/L)	Susceptibility %	Range (mg/L)	Susceptibility %
Amikacin	1-4	0	1-2	100
Ampicillin/Sulbactam	16 >32	0	⩽0.25-1	100
Ciprofloxacin	⩽0.031-> 4	50	⩽0.063-⩽0.031	100
Cefotaxim	>8	0	⩽0.063	100
Cefuroxim	>32	0	⩽0.25-2	67
Gentamycin	0.25-1	50	-	-
Imipenem	1-8	50	0.25-2	100
Meropenem	⩽0.125- 0.25	100	⩽0.125	100
Moxifloxacin	0.125->4	50	0.25-⩽0.031	100
Pieracillin	1	100	⩽0.50	100
Cotrmoxzol	0.5->16	50	4->16	0
Ampicillin	>32	0	0.5-2	100
Aztronam	4->16	0	⩽0.25	100
Ceftazidim	4	0	⩽0.25	100
Ceftibuten	1—2	50	⩽0.031	100
Colistin	>8	0	>8	0
Ertapenem	⩽0.031-0.5	50	⩽0.031	100
Doripenem	⩽0.125-8	50	⩽0.125-0.25	100
Fosfomycin	16-128	50	16-128	67
Levofloxacin	⩽0.063-0.5	100	⩽0.063	100
Piperacillin	64->64	0	⩽0.5	100
Tobramycin	0.5-1	100	0.25	100
Roundup (mg/mL)	2.4	100	2.4	100

higher resistance levels. Moreover, a clinically occurring ESBL-resistance is often linked to conjugative plasmids (Li et al., 2019), which provides another path to connect genotypic ESBL production with Roundup tolerance. Recently, Liao and co-workers highlighted the role of glyphosate in the emergence of antimicrobial resistance in agricultural environments by enrichment of the antibiotic resistance genes and mobile genetic elements in soil microbiomes (Liao et al., 2021).

In our investigation, the Mut-A exhibited a significant increase in the MIC value for both ampicillin alone and ampicillin/sulbactam combination (Table 6). However, this mutant exhibited a much smaller increase in MIC value towards piperacillin/tazobactam combination versus piperacillin alone. The *E. coli* B253 is not ESBL-producing bacteria, so the ß-lactamase inhibitor tazobactam should have a negligible effect on this strain compared to the effect

Table 6: Antibiotic sensitivity of glyphosate mutants of the non-ESBL *E. coli* (B253)

Antibiotics	Wild	Mut-A	Mut-N	Mut-F	Mut-2	Mut-3	Mut-5	Mut-6	Mut-8	Mut-32
Amikacin	1	1	1	1	1	1	1	1	1	1
Ampicillin-Sulbactam	1	>32	4	1	2	1	2	2	2	1
Ciprofloxacin	⩽0.031	⩽0.031	⩽0.031	⩽0.031	⩽0.031	⩽0.031	⩽0.031	⩽0.031	⩽0.031	⩽0.031
Cefotaxim	⩽0.063	⩽0.125	⩽0.125	⩽0.063	⩽0.063	⩽0.063	⩽0.063	0.125	⩽0.063	⩽0.063
Cefuroxim	2	8	8	2	4	2	4	8	2	4
Doxycyclin	1	>8	1	1	2	1	2	2	1	1
Gentamycin	0.25	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Imipenem	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125
Meroppenem	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125
Moxifloxacin	⩽0.031	⩽0.031	⩽0.063	⩽0.031	⩽0.031	⩽0.031	⩽0.031	⩽0.031	⩽0.031	⩽0.031
Piperacillin-Tazobactam	1	4	4	1	1	1	2	2	1	2
Cotrimoxazol (Sulf./Trim)	⩽0.125	>16	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125
Ampicillin	4	>32	8	4	4	4	4	8	4	4
Aztreonam	⩽0.25	⩽0.25	⩽0.25	⩽0.25	⩽0.25	⩽0.25	⩽0.25	⩽0.25	⩽0.25	⩽0.25
Ceftazidim	⩽0.25	⩽0.25	⩽0.25	⩽0.25	⩽0.25	⩽0.25	⩽0.25	⩽0.25	⩽0.25	⩽0.25
Ceftibuten	0.5	0.5	0.25	0.125	0.125	0.125	0.25	0.125	0.0625	0.125
Colistin	0.25	0.25	0.5	0.5	0.25	0.5v 0.5	0.25	0.25	0.25	
Brtapenem	⩽0.031	⩽0.031	⩽0.031	⩽0.031	⩽0.031	⩽0.031	⩽0.031	⩽0.031	⩽0.031	⩽0.031
Doripenem	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125	⩽0.125
Fosfomycin	8	8	8	32	2	8	8	16	16	32
Levofloxacin	⩽0.063	⩽0.063	⩽0.063	⩽0.063	⩽0.063	⩽0.063	⩽0.063	⩽0.063	⩽0.063	⩽0.063
Piperacillin	1	32	4	2	2	2	2	2	1	2
Tobramycin	0.5	0.5	0.25	0.25	0.5	0.5	0.5	0.5	0.5	0.25

of piperacillin alone (Paterson and Bonomo, 2005). This difference in MIC values is still not fully understood. Altogether, our results show that ESBL-producing *Enterobacteriaceae* exhibited a higher tolerance to Roundup than ESBL-negative *Enterobacteriaceae*, highlighting the urgent need for further investigation of the potential link between antibiotic resistance and the herbicide glyphosate.

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