Microbiology Research Journal International

31(5): 64-71, 2021; Article no.MRJI.71955
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Susceptibility to Antibiotics and Reactive Oxygen Species in *Escherichia coli*: A Survey of Clinical and Environmental Isolates

Carlos F. Amábile-Cuevas[1*], Leticia Martínez[2] and Irma Rosas[2]

[1]Fundación Lusara, Mexico City, Mexico.
[2]Centro de Ciencias de la Atmósfera, Universidad Nacional Autónoma de México, Mexico City, Mexico.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2021/v31i530321
Editor(s):
(1) Dr. Ana Cláudia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) P. N. Remya, India.
(2) Devasish Bose, Dr. Harisingh Gour University, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/71955

Original Research Article

Received 01 June 2021
Accepted 05 August 2021
Published 09 August 2021

ABSTRACT

Aims: Some bacterial responses to oxidative stress also diminish antibiotic susceptibility; also, some antibiotics do increase oxidative stress within bacterial cells. Linkage or cross-resistance to prooxidants and antibiotics could facilitate the selection of antibiotic resistance and/or virulence. We made this survey in order to detect this possible linkage in *Escherichia coli* isolates.
Methodology: The susceptibility of 102 *E. coli* clinical (causative of urinary or gastrointestinal infections) and environmental (rural or urban dust) isolates towards paraquat, H_2O_2, and antibiotics was measured using disc assays. Catalase and superoxide-dismutase (SOD) activities were measured.
Results: Susceptibility to prooxidants was similar across isolates of all four sources, but urinary and urban dust isolates were more resistant to antibiotics. H_2O_2 "resistant" organisms had more antibiotic resistance phenotypes, particularly towards sulfadiazine and tetracycline. Paraquat "resistance" seems associated to beta-lactam resistance; but paraquat "susceptibility" seems associated to resistance towards chloramphenicol, gentamicin, ciprofloxacin and nitrofurantoin.

**Corresponding author: E-mail: carlos.amabile@lusara.org*

Prooxidant disc assays correlate to catalase and superoxide-dismutase activities. A weak relationship H_2O_2/antibiotic-resistance, but not superoxide/antibiotic-resistance, is suggested.
Conclusion: Overall, antibiotics exerting their action through oxidative stress, do not seem to have resulted in the co-selection of oxidative stress resistance, or vice versa. However, a possible link between resistance to some antibiotics and to H_2O_2 might contribute to co-selection between these two chemical insults.

Keywords: Superoxide; hydrogen peroxide; oxidative stress; antibiotic resistance.

1. INTRODUCTION

The linkage between bacterial susceptibility towards antibiotics, and towards some reactive oxygen species (ROS; particularly superoxide, $O_2^{\cdot-}$, and hydrogen peroxide, H_2O_2), have been explored extensively. On the one hand, some responses to oxidative stress, such as the one governed by the *soxRS* genes in *E. coli* and other gram-negative bacteria, include mechanisms that reduce the activity of several antibiotics. This is accomplished mainly by a diminished cytoplasmic accumulation of the drugs both, by reduced permeability and by increased efflux. Strains lacking these regulatory genes are more susceptible to several antibiotics, while mutants constitutively expressing the *soxRS* regulon are less susceptible to antibiotics than their wild-type counterparts [1,2]. This has been documented in other bacterial species (*e.g.*, [3]). Similarly, the bacterial response governed by OxyR towards H_2O_2 and related oxidative stress, also regulates antibiotic resistance in *E. coli* and other gram-negative bacteria [4]. On the other hand, a number of papers have reported that some antibiotics, especially those deemed "bactericidal", increase the intracellular production of ROS [5], up to the point that this has been proposed to be the actual mechanism of their antimicrobial action [6]. Although this does not seem to be the case [7], some antibiotics are likely to increase the levels of ROS within the bacterial cell, perhaps contributing to the overall stress during antibiotic exposure [8]. It is therefore possible that increased resistance towards ROS might confer some protection against antibiotics that produce oxidative stress. As bacteria, both as human commensal/pathogens (*e.g.*, [9]), or in the open environment (*e.g.*, [10,11]), commonly face prooxidants and antibiotics, mechanisms that protect against both may have potential repercussions in the efficacy of antibiotic treatments. Most of these observations, however, come from laboratory strains and conditions, but little is known about the occurrence of such phenomena in clinical or environmental settings. Here, the activity of representative antibiotics, and known sources of ROS (H_2O_2 and paraquat, a known generator of intracellular $O_2^{\cdot-}$), was tested against a group of clinical and environmental *E. coli* isolates. This is, to our knowledge, the first attempt at co-relating the susceptibility towards prooxidants and antibiotics, in a set of isolates from very diverse origin.

2. MATERIALS AND METHODS

2.1 Isolates

A total of 102 *E. coli* isolates were included: 24 causative of community-acquired urinary tract infection (Uri); 21 from fecal samples from patients with diarrhea, where the isolate was deemed causative of the illness (Fec); 27 from outdoor rural dust (RD), collected at the Mezquital Valley, close to irrigation canals receiving raw wastewater from Mexico City; and 30 from urban dust (UD) collected at Mexico City. All organisms were identified using standard biochemical methods, and kept in glycerol-containing (25%) liquid media, under liquid nitrogen.

2.2 Antibiotic and Prooxidant Susceptibility Assays

Susceptibility towards ampicillin, amoxicillin-clavulanate, cefotaxime, sulfadiazine, chloramphenicol, tetracycline, gentamicin, ciprofloxacin and nitrofurantoin, was assessed by the method of disc diffusion on Mueller-Hinton agar plates [12] and using commercially-available antibiotic discs (BBL, 10, 20/10, 30, 250, 30, 30, 10, 5 and 300 µg, respectively). Susceptibility to paraquat and hydrogen peroxide was also assessed by disc diffusion, plating ~$5x10^8$ CFU of each strain on LB agar plates, and then filter paper discs containing either 400 µg of paraquat (PQ, Sigma), or 8.8 µmol of H_2O_2 (freshly prepared by dispensing concentrated solution, Sigma) were placed on top [13];

inhibitory halos around each disc were measured after a 35 ºC/overnight incubation.

2.3 Catalase and Superoxide-dismutase Activity Assays

Catalase activity was measured by a "foamometric" assay [14], where ~1x10^9 cells from overnight cultures, in 100 µL distilled water, (with or without further incubation at 55 ºC/15 min, to distinguish heat-labile HPI encoded by *katG*, from heat-stable HPII encoded by *katE*) were mixed with 100 µL 1% triton X and 100 µL 30% H_2O_2, and the height of the foam column generated by the oxygen bubbles was measured after a 10 min incubation at room temperature, and compared to a calibration curve performed using known concentrations of bovine liver catalase (Sigma). Superoxide-dismutase (SOD) activity was semi-quantified using SOD activity gels [15], running crude extracts obtained by mixing 0.1-mm zirconium beads with pelleted mid-exponential phase cells suspended in 50 mM Tris, 0.2 M NaCl, pH 7.5 buffer.

3. RESULTS

3.1 Susceptibility to Antibiotics and Prooxidants

Overall results of oxidants and antibiotics susceptibility are shown in Fig. 1. Table 1 contains average data of inhibitory halos around discs containing PQ or H_2O_2, and number of antibiotic resistance phenotypes. Fec isolates had PQ halos 11% larger than average; UD isolates had H_2O_2 halos 11% larger than average; and Uri and UD isolates were resistant to more antibiotics than Fec and RD isolates.

3.2 Prooxidant and Antibiotic Resistant and Susceptible Organisms

In order to simplify the analysis of the data, organisms were deemed "resistant" if inhibitory halos of oxidants were below one standard deviation (SD) of the global averages, or above one SD of the average number of resistances; and "susceptible" if halos were one SD above, and number of resistances were one SD below the global averages (Table 2). Both PQ^R and PQ^S organisms were more resistant to antibiotics (13% and 27%, respectively, measured as the difference in the average number of resistance phenotypes), while $H_2O_2{}^R$ were 20% more resistant to antibiotics, compared to the total average. Among Uri isolates, susceptibility to PQ, H_2O_2 and antibiotics are less common than average; among Fec isolates, PQ susceptibility is more common, and H_2O_2 resistance is less common than average; among RD isolates, PQ, H_2O_2 and antibiotics' resistance are more common than average, while the opposite results were observed from UD isolates.

3.3 Individual Antibiotics and Prooxidant Susceptibility

Results for individual antibiotics are shown in Table 3. Resistance to PQ seems linked to resistance to ampicillin and amoxicillin-clavulanate (tetracycline resistance is more common among both, PQ^R and PQ^S subgroups); while susceptibility to PQ seems linked to resistance to chloramphenicol, gentamicin, tetracycline, nitrofurantoin and, particularly, to ciprofloxacin. Resistance to H_2O_2 seems linked to resistance to sulfadiazine and tetracycline (chloramphenicol resistance is more common among both, $H_2O_2{}^R$ and $H_2O_2{}^S$ subgroups). Uri isolates are particularly more resistant to ampicillin and amoxicillin-clavulanate, than the global average; and UD isolates are more resistant to chloramphenicol, gentamicin, nitrofurantoin, ciprofloxacin and sulfadiazine, than the average.

3.4 Catalase and SOD Activities and Prooxidant Disc Assays

Susceptibility to H_2O_2, measured as inhibitory halos in the disc assay, did correlate with production of catalase HPII encoded by *katE*. Average activity per 1x10^9 cells among $H_2O_2{}^R$ isolates was 43.4 (SD 22.9) units of HPI and 44.3 (SD 18.7) units of HPII; while among $H_2O_2{}^S$ isolates was 31.4 (SD 31.4; $p = 0.402$, Student *t* test) units of HPI, and 7.7 (SD 9.7; $p = 0.002$) units of HPII. On the other hand, susceptibility to paraquat in the disc assay showed that, while resistant isolates have a uniform SOD activity, susceptible ones had a diversity of SOD profiles, especially affecting the inducible, Mn-SOD (Fig. 2).

4. DISCUSSION

Overall, a strong correlation between resistance to oxidative stress and antibiotics was not found. Isolates able to withstand higher concentrations of H_2O_2 seem to also be able to resist the effect of more antibiotics (a 20% increase); however,

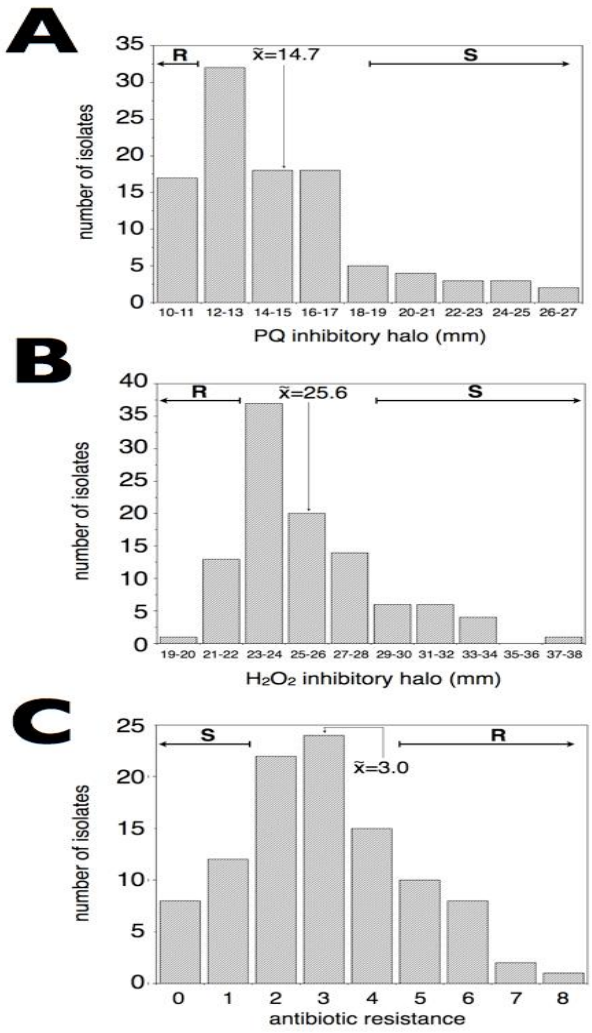

Fig. 1. Susceptibility distribution of clinical and environmental *E. coli* isolates towards prooxidants and antibiotics. Inhibitory halos in mm around discs containing paraquat (A) or H_2O_2 (B), and number of antibiotic resistance phenotypes per isolate (C) are shown in each panel, along with the global average. Organisms with values one standard deviation above or below average were considered susceptible (S) or resistant (R), respectively (except for antibiotic resistance)

Table 1. Susceptibility towards oxidants and antibiotics among clinical and environmental isolates of *E. coli*.

	total	Uri		Fec		RD		UD	
	$\bar{x}$(SD)	$\bar{x}$(SD)	ratio	$\bar{x}$(SD)	ratio	$\bar{x}$(SD)	ratio	$\bar{x}$(SD)	ratio
PQ	14.7(3.8)	13.5(2.4)	0.92	16.3(4.4)	**1.11**	13.5(3.0)	0.92	15.7(4.3)	1.07
H_2O_2	25.6(3.4)	24.3(1.5)	0.95	25.8(2.8)	1.01	23.3(2.6)	0.91	28.4(3.4)	**1.11**
ATB	3.0(1.8)	3.5(1.6)	**1.17**	2.2(1.6)	**0.73**	2.5(1.7)	**0.83**	3.7(1.9)	**1.23**

Data are: averages (x) with standard deviations (SD) of inhibitory halos, in mm, for PQ and H_2O_2; and of number of antibiotic resistances (ATB). Uri, isolates from urinary infections; Fec, isolates from diarrheal samples; RD, isolates from rural dust; UD, isolates from urban dust. Ratio is the quotient between each subgroup value and the total; those with a difference ≥10% are in bold.

Table 2. Susceptibility towards oxidants and antibiotics among clinical and environmental isolates of *E. coli*.

	PQ		H_2O_2		nATB		Uri		Fec		RD		UD	
	$\bar{x}$(SD)	ratio	$\bar{x}$(SD)	ratio	$\bar{x}$(SD)	ratio	n(%)	dif	n(%)	dif	n(%)	dif	n(%)	dif
Total	14.7(3.8)		25.6(3.4)		3.0(1.8)		24		21		27		30	
PQ^R	*10.6(0.5)*	*0.72*	25.1(3.4)	0.98	3.4(1.7)	**1.13**	5(29)	+5	2(12)	-9	8(47)	**+20**	1(6)	**-24**
PQ^S	*22.6(2.6)*	*1.54*	27.1(4.0)	1.06	3.8(1.6)	**1.27**	1(8)	**-16**	5(38)	**+17**	1(8)	**-19**	6(46)	**+16**
$H_2O_2{}^R$	14.3(3.5)	0.97	*21.7(0.6)*	*0.85*	3.6(2.1)	**1.20**	2(14)	**-10**	1(7)	**-14**	10(71)	**+44**	1(7)	**-23**
$H_2O_2{}^S$	15.2(4.7)	1.03	*31.7(2.1)*	*1.24*	2.8(1.6)	0.93	0	**-24**	3(18)	-3	1(6)	**-21**	13(76)	**+46**
ATB^R	15.2(3.5)	1.03	25.2(2.5)	0.98	*5.7(0.8)*	*1.9*	5(23)	-1	4(19)	-2	3(14)	**-13**	9(43)	**+13**
ATB^S	14.3(3.3)	0.97	25.3(3.2)	0.99	*0.6(0.5)*	*0.21*	1(5)	**-19**	6(30)	+9	9(45)	**+18**	4(20)	**-10**

Data are: averages ($\bar{x}$) with standard deviations (SD) of diameter of inhibitory halos, in mm, for paraquat (PQ) or hydrogen peroxide (H_2O_2), or of number of antibiotic resistance phenotypes (nATB), for subpopulations deemed "susceptible" (S) or "resistant" (R; see text). Ratio is the quotient of each group's average and total average; or the number of isolates (n) of each source, and the percentage (%) for each group; and the difference (dif) between each group's percentage and the total percentage. Ratio or dif values with a difference ≥10% are in bold

Table 3. Resistance towards individual antibiotics, among oxidants' resistant and susceptible subgroups, and among each source subgroup

	AM		AMC		CTX		SD		C		GM		TE		CIP		FM	
	n(%)	dif	n(%)	dif	n(%)	dif	n(%)	dif	n(%)	dif	n(%)	dif	n(%)	dif	n(%)	dif	n(%)	dif
total	55		9		2		80		42		13		71		24		11	
PQ^R	13(76)	**+21**	4(24)	**+15**	0	-2	14(82)	+2	6(35)	-7	2(12)	-1	15(88)	**+17**	3(18)	-6	1(6)	-5
PQ^S	7(54)	-1	0	-9	0	-2	11(85)	+5	7(54)	**+12**	3(23)	**+10**	11(85)	**+14**	6(46)	**+22**	3(23)	**+12**
$H_2O_2{}^R$	7(50)	-5	1(7)	-2	1(7)	+5	13(93)	**+13**	8(57)	**+15**	3(21)	+8	13(93)	**+22**	3(21)	-3	2(14)	+3
$H_2O_2{}^S$	8(47)	-8	1(6)	-3	0	-2	14(82)	-2	11(65)	**+23**	2(12)	-1	8(47)	**-24**	2(12)	**-12**	3(18)	+7
Uri	22(92)	**+37**	7(29)	**+20**	2(8)	+6	20(83)	+3	3(13)	**-29**	3(13)	0	18(75)	+4	6(25)	+1	1(4)	-7
Fec	8(38)	**-17**	1(5)	-4	0	-2	15(71)	-9	4(19)	**-23**	2(10)	-3	13(62)	-9	2(10)	**-14**	2(10)	-1
RD	10(37)	**-18**	0	-9	0	-2	18(67)	**-13**	11(41)	-1	2(7)	-6	22(81)	**-10**	5(19)	-5	0	**-11**
UD	15(50)	-5	1(3)	-6	0	-2	27(90)	**+10**	25(83)	**+41**	6(20)	**+17**	18(60)	**-11**	11(37)	**+13**	8(27)	**+16**

AM, ampicillin; AMC, amoxicillin-clavulanate; CTX, cefotaxime; SD, sulfadiazine; C, chloramphenicol; GM, gentamicin; TE, tetracycline; CIP, ciprofloxacin; and FM, nitrofurantoin.

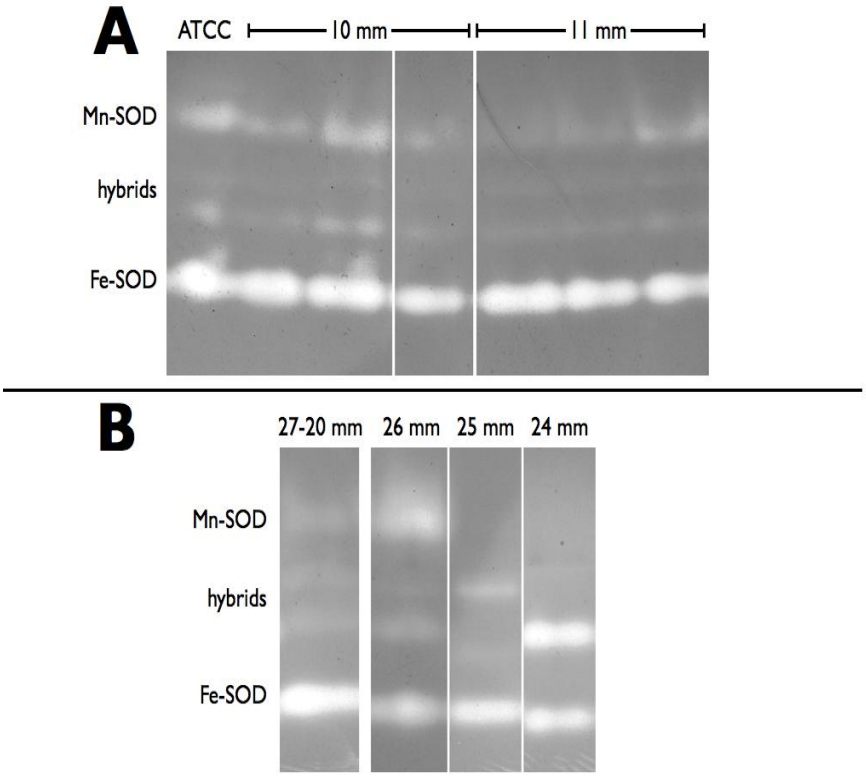

Fig. 2. Superoxide dismutase activity of PQ^R and PQ^S isolates

Superoxide dismutase (SOD) activity gels are shown for organisms considered "resistant" (A) or "susceptible" (B) to paraquat; the enzyme activity is revealed as transparent bands within a dark background, with Fe-SOD being a constitutive enzyme, Mn-SOD an inducible one, and faint hybrids between them. Strain ATCC 25922 was included as a wild-type control, and activity of some representatives of each group, labeled as the diameter of the inhibitory halo around the paraquat disc are shown. "Resistant" organisms had a very uniform activity, while a diversity of SOD profiles was observed in "susceptible" organisms.

individual antibiotic-resistance phenotypes associated to H_2O_2-resistance, sulfadiazine and tetracycline are considered as "bacteriostatic" agents. Regarding paraquat, it was the most susceptible isolates the ones with higher number of antibiotic resistance phenotypes, including the bactericidal fluoroquinolone ciprofloxacin and, to a lesser extent, gentamicin and chloramphenicol. The activity of nitrofurantoin has been shown to depend on an enzyme which expression is governed by the *soxRS* genes, hence it was an expected outcome to find slightly more resistance to the drug amongst the PQ^S isolates [16]. Perhaps the results from the environmental isolates can better summarize these findings: while amongst RD isolates there are more paraquat- and H_2O_2-resistant isolates, they have fewer antibiotic resistance phenotypes; on the contrary, there were more prooxidant-susceptible isolates from UD, but those have more resistance phenotypes. Whatever the conditions that are causing these environmental isolates to resist more or less to prooxidants or antibiotics, both features do not seem to be linked.

The linkage between resistance towards superoxide-generating agents, such as PQ, and diminished susceptibility to several antibiotics, have been shown in laboratory conditions, in *E. coli* and other gram-negative bacteria (*e.g.*, [17]). However, such a linkage was not found, and perhaps even just the opposite. While reduced accumulation –the main mechanism of antibiotic resistance mediated by *soxRS*, is a successful strategy in the short term, the "trade-offs" might end up reducing fitness [18]. On the other hand, the weak correlation between H_2O_2- and antibiotic-resistance might be indicative of a more direct association of anti-oxidant defenses in protecting against the effects of antibiotics.

Nevertheless, being the two antibiotic-resistance phenotypes with the strongest linkage to H_2O_2-resistance anything but bactericidal (*i.e.*, sulfadiazine and tetracycline), the role of ROS is not that clear. The exposure of *E. coli* to sulfamethoxazole was recently shown to induce a metabolic pathway that produces antioxidant pterin-phenylpyruvate conjugates [19] suggesting that sulfonamide antibiotics do exert oxidative stress. In any case, the likelihood of some antibiotics fostering or co-selecting for H_2O_2-resistance could link the use of those antibiotics to an increased bacterial virulence, as oxidative-stress responses have a known role as pathogenic determinants [20].

The method used here to assess antibiotic susceptibility is a coarse one, only capable of distinguishing between susceptibility and resistance by using clinical breakpoints. It is possible that oxidant-resistant isolates have diminished susceptibility but not up to the point of being fully-resistant. While the method lacks the resolution needed, the average size of inhibitory halos among susceptible isolates do not differ between prooxidant-susceptible and -resistant strains (not shown). The method for assessing susceptibility towards H_2O_2 and paraquat is equally coarse, unable to distinguish the underlying mechanism of purported susceptibility or resistance. While there was a correlation between the sizes of inhibitory halos, and the activities of enzymes inactivating respective ROS, very different genotypes could have been grouped under the same category, possibly confounding the associations. Further analyses, utilizing more precise and specific molecular methods to assess the existence and extent of the linkage between prooxidant- and antibiotic-resistance.

5. CONCLUSION

The proposed oxidative stress proposed as secondary “mechanism of action” for some antibiotics, do not seem to have resulted in the co-selection of oxidative stress resistance, or vice versa, in clinical and environmental isolates of *E. coli*. Weak linkage between H_2O_2-resistance and antibiotic multi-resistance was found, and resistance to sulfonamides and tetracycline seem to contribute especially to this effect. Resistance to $O_2^{\cdot-}$-generator paraquat seems only clearly related to resistance to aminopenicillins. In any case, a possible link between resistance to some antibiotics and to prooxidants could contribute to co-selection.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Amábile-Cuevas CF, Demple B. Molecular characterization of the *soxRS* genes of *Escherichia coli*: two genes control a superoxide stress regulon. Nucleic Acids Res. 1991;19:4479-84.
2. Demple B, Amábile-Cuevas CF. Multiple resistance mediated by individual genetic loci. In: Amábile-Cuevas CF, editor. Multiple drug resistant bacteria. Wymondham: Horizon; 2003.
3. Telke AA, Olaitan AO, Morand S, Rolain JM. *soxRS* induces colistin hetero-resistance in *Enterobacter asburiae* and *Enterobacter cloacae* by regulating the *acrAB-tolC* efflux pump. J Antimicrob Chemother. 2017;72:2715-21.
4. Srinivasan VB, Mondal A, Venkataramaiah M, Chauhan NK, Rajamohan G. Role of $oxyR^{KP}$, a novel LysR-family transcriptional regulator, in antimicrobial resistance and virulence in *Klebsiella pneumoniae*. Microbiology. 2013;159:1301-14.
5. Tkachenko AG, Akhova AV, Shumkov MS, Nesterova LY. Polyamines reduce oxidative stress in *Escherichia coli* cells exposed to bactericidal antibiotics. Res Microbiol. 2012;163:83-91.
6. Dwyer DJ, Belenky PA, Yang JH, MacDonald IC, Martell JD, Takahashi N, Chan CTY, Lobritz MA, Braff D, Schwarz EG, Ye JD, Pati M, Vercruysse M, Ralifo PS, Allison KR, Khalil AS, Ting AY, Walker GC, Collins JJ. Antibiotics induce redox-related physiological alterations as part of their lethality. Proc Natl Acad Sci USA. 2014;111:e2100-9.

7. Mahoney TF, Silhavy TJ. The Cpx stress response confers resistance to some, but not all, bactericidal antibiotics. J Bacteriol. 2013;195:1869-74.
8. Kim SY, Park C, Jang HJ, Kim BO, Bae HW, Chung IY, Kim ES, Cho YH. Antibacterial strategies inspired by the oxidative stress and response networks. J. Microbiol. 2019;57:203-12.
9. Yamashita K, Miyoshi T, Arai T, Endo N, Itoh H, Makino K, Mizugishi K, Uchiyama T, Sasada M, Ozone production by amino acids contributes to killing of bacteria. Proc Natl Acad Sci USA. 2008;105:16912-17.
10. Amábile-Cuevas CF. Antibiotics and antibiotic resistance in the environment, London: CRC Press; 2016.
11. Zhang T, Shi XC, Xia Y, Mai L, Tremblay PL. Escherichia coli adaptation and response to exposure to heavy atmospheric pollution. Sci Rep. 2019;9: 10879.
12. CLSI. Performance standards for antimicrobial susceptibility testing, 30th ed. Wayne: Clinical and Laboratory Standards Institute; 2020.
13. Greenberg JT, Demple B. Overproduction of peroxide-scavenging enzymes in *Escherichia coli* supresses spontaneous mutagenesis and sensitivity to redox-cycling agents in *oxyR*$^{-}$ mutants EMBO J. 1988;7:2611-7.
14. Iwase T, Tajima A, Sugimoto S, Okuda K, Hironaka I, Kamata Y, Takada K, Mizunoe Y. A simple assay for measuring catalase activity: a visual approach, Sci Rep. 2013; 3:3081.
15. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem. 1971;44:276-87.
16. Amábile-Cuevas CF, Arredondo-García JL. Nitrofurantoin, phenazopyridine, and the superoxide-response regulon *soxRS* of *Escherichia coli*. J Infect Chemother. 2013; 19:1135-40.
17. Martins D, McKay GA, English AM, Nguyen, D. Sublethal paraquat confers multidrug tolerance in *Pseudomonas aeruginosa* by inducing superoxide dismutase activity and lowering envelope permeability. Front Microbiol. 2020; 11:576708.
18. Holden ER, Webber MA. MarA, RamA, and SoxR as mediators of stress response: survival at a cost. Front Microbiol. 2020; 11:828.
19. Park HB, Wei Z, Oh J, Xu H, Kim CS, Wang R, Wyche TP, Piizzi G, Flavell RA, Crawford JM. Sulfamethoxazole drug stress upregulates antioxidant immunom-odulatory metabolites in *Escherichia coli*. Nat Microbiol. 2020;5: 1319-29.
20. Johnson JR, Clabots C, Rosen H. Effect of inactivation of the global oxidative stress regulator *oxyR* on the colonization ability of *Escherichia coli* O1:K1:H7 in a mouse model of ascending urinary tract infection. Infect Immun. 2006;74:461-8.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/71955