Exposure to clinical X-ray radiation does not alter antibiotic susceptibility or genotype profile in Gram-negative and Gram-positive clinical pathogens

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ABSTRACT

Inadvertent exposure of bacterial pathogens to X-ray radiation may be an environmental stress, where the bacterium may respond by increasing mutational events, thereby potentially resulting in increased antibiotic resistance and alteration to genotypic profile. In order to examine this, four clinical pathogens, including the Gram-negative organisms *Escherichia coli* O157:H7 NCTC12900 and *Pseudomonas aeruginosa* NCTC10662, as well as the Gram-positive organisms *Staphylococcus aureus* NCTC6571 and *Enterococcus faecium* were exposed to X-rays (35,495 cGy/cm^2) over a seven-day period. Antibiotic susceptibility was assessed before, during and after exposure by examining susceptibility, as quantified by E-test with six antibiotics, as well as to a further 11 antibiotics by measurement of susceptibility zone sizes (mm). Additionally, the DNA profile of each organism was compared before, during and after exposure employing the enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC PCR). Results indicated that exposure of these organisms to this amount of X-ray radiation did not alter their antibiotic susceptibility, nor their genomic DNA profile. Overall, these data indicate that exposure of bacteria to X-ray radiation does not alter the test organisms' antibiotic susceptibility profiles, nor alter genomic DNA profiles of bacteria, which therefore does not compromise molecular epidemiological tracking of bacteria within healthcare environments in which patients have been exposed to X-ray radiation.

KEY WORDS: Anti-bacterial agents.
Genotyping techniques.
Gram-negative bacteria.
Gram-positive bacteria.
Microbial sensitivity tests.
X-rays.

Introduction

Recent published data from this group[1] and others[2] have demonstrated that when bacteria are exposed to environmental stresses, such as altered pH, heat shock and increased salinity, they are able to alter their antibiotic susceptibility by becoming either more susceptible to antibiotic agents or more resistant to antibiotics. For example, when the authors sublethally stressed *Staphylococcus aureus* organisms with increased salt or pH, up to a four-fold increase in minimum inhibitory concentration (MIC) was observed with gentamicin and erythromycin.[1]

X-ray radiation has the ability to kill bacterial organisms depending on the dose absorbed by target bacterial cells, mainly through DNA damage inducing strand breaks.[3] Therefore, such radiation has the probability of inducing mutations, ranging from mild and 'silent' mutation through to catastrophic mutations, leading to terminal events within the bacteria.

In nature, mutagenesis is hypothesised to play roles in adaptation and subsequent propagation via chromosomal rearrangement, alteration in target sites on cells, and by deregulation of enzyme synthesis.[4,5] What has not been examined to date is the effect of X-ray radiation on clinical pathogens carried in/on patients being examined radiologically, particularly in terms of the bacteria's susceptibility to antibiotics, as well as the organism's ability to modify its genome, thus generating new DNA fingerprints.

Therefore, it is important to examine the effect of exposure to X-ray radiation on the bacterial stress response. Thus, the aim of this study is to examine the effect of clinical X-ray exposure on the antibiotic susceptibility of four bacterial pathogens in order to determine if such radiation at clinical levels increases antibiotic resistance, as well as assess its ability to cause alterations in the bacterial genome, thereby altering the organism's genotype profile.

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Materials and methods

Bacterial isolates

Four bacterial reference isolates were used in this study, including two Gram-positive organisms, *Staphylococcus aureus* NCTC6571 and *Enterococcus faecium*, as well as two Gram-negative organisms, *Escherichia coli* O157:H7 NCTC

12900 and *Pseudomonas aeruginosa* NCTC10662. These isolates were part of the Northern Ireland Public Health Laboratory (NIPHL) Strain Repository and were recovered from storage at –80°C. All isolates were subcultured at least three times on Columbia blood agar (CM0331, Oxoid, Basingstoke, UK), supplemented with 5% (v/v) defibrinated horse blood for 24 h at 37°C under aerobic conditions. Careful attention was given to purifying the isolates from single colony picks on at least three occasions, to ensure study of a single clonal type of each organism in downstream analyses.

Sublethal X-ray irradiation

Fresh (24 h) cultures of all organisms were subcultured separately on fresh Columbia blood agar (Oxoid) supplemented with 5% (v/v) defibrinated horse blood. A fresh 0.5 McFarland standard was prepared separately for all four organisms in quarter-strength Ringer's solution (Oxoid, BR0049G) and 100 µL bacterial suspension was added to tryptone soya broth (TSB, 20 mL, Oxoid CM0129) in thin-walled sterile plastic containers. Inoculated containers were placed at ambient temperature in a location adjacent to that in which patients were being screened in the X-ray screening room at the Department of Radiology, Belfast City Hospital, for a period of seven days. The X-ray dose of every event was logged over this period and the total radiation determined at the completion of this period (35,495 cGy). Organisms were selected for further downstream processing and three chronological time points were established, including i) before exposure to X-rays, ii) during exposure to X-rays for seven days (without subculture) and iii) after X-ray exposure (i.e., the first passaged cells following completion of X-ray exposure).

Determination of antimicrobial susceptibility

Antibiotic susceptibility testing was performed in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines[6] using i) standard disk susceptibility testing on all isolates at each of the three time points above, measuring inhibition zone sizes (mm) to the following 11 antibiotics: amoxicillin with clavulanic acid (3 µg), cefotaxime (30 µg), chloramphenicol (10 µg), clindamycin (2 µg), colistin (10 µg), erythromycin (5 µg), fusidic acid (10 µg), penicillin (2 µg), rifampicin (5 µg), teicoplanin (30 µg) and tetracycline (10 µg); and ii) quantitative MIC (µg/mL) susceptibility data was obtained by examination with the following E-tests (bioMérieux, Basingstoke, Hampshire, UK): cefotaxime, ciprofloxacin, ceftazidime, ceftriaxone, piperacillin and trovafloxacin. Briefly, in both cases, a cotton swab was charged with inoculum equivalent to a 0.5 McFarland standard, which was inoculated on the surface of Columbia blood agar (Oxoid, CM0331), supplemented with 5% (v/v) defibrinated horse blood. On drying, a standard disk-diffusion assay was performed with either an E-test strip or a susceptibility disk. Plates were

Table 1. Comparison of inhibition zone sizes with 11 antibiotic agents tested against four organisms before, during and after exposure to X-ray radiation.

		Size of inhibition zone (mm)										
		Amoxicillin-clavulanic acid (3 µg)	Clindamycin (2 µg)	Cefotaxime (30 µg)	Chloramphenicol (30 µg)	Colistin (10 µg)	Erythromycin (5 µg)	Penicillin (2 µg)	Fusidic acid (10 µg)	Rifampicin (5 µg)	Teicoplanin (30 µg)	Tetracycline (10 µg)
Staphylococcus aureus NCTC6571	Before	40	24	30	30	20	26	34	24	35	18	28
	During	30	24	34	28	20	24	32	24	30	16	30
	After	30	25	30	24	16	25	30	20	32	16	30
Escherichia coli NCTC12900	Before	10	0	30	20	20	0	0	0	10	0	20
	During	10	0	34	18	15	0	0	0	10	0	18
	After	10	0	30	20	18	0	0	0	10	0	16
Pseudomonas aeruginosa NCTC10662	Before	0	0	24	15	22	0	0	0	0	0	10
	During	0	0	20	25	15	0	0	0	0	0	8
	After	0	0	24	20	23	0	0	0	0	0	8
Enterococcus faecium	Before	22	8	30	22	12	20	12	12	15	20	25
	During	22	8	35	25	15	20	10	15	15	20	24
	After	24	8	30	25	15	20	10	15	10	18	24

Before: analysis of antibiotic susceptibility before X-ray exposure.

During: analysis of antibiotic susceptibility at the end of X-ray exposure.

After: analysis of antibiotic susceptibility on first passaged cells following completion of X-ray exposure.

incubated aerobically at 37°C for 24 h prior to reading. In the case of disk susceptibility testing, the diameter of the zone of inhibition was measured (mm) manually and the MIC determined in the case of E-tests, as instructed by the manufacturer.

Determination of whole genome DNA profile

Genomic DNA was subjected to the enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR), as previously described.[7] Briefly, PCR reaction mixes (25 µL) contained 2 µL DNA template (50 ng DNA, 10 mmol/L Tris-HCl [pH 8.3], 50 mmol/L KCl, 1.8 mmol/L $MgCl_2$, 200 µmol/L each dNTP, 1.5 units *Thermus aquaticus* [*Taq*] DNA polymerase [New England Biolabs, Hertfordshire, UK] and 100 µmol/L each primer). All DNA isolation procedures were carried out in accordance with the DNA contamination management guidelines of Millar *et al*.[8] in a Class II Biological Safety Cabinet (MicroFlow, England).

Extracted DNA was transferred to a clean tube and stored at –80°C prior to PCR amplification. The primers ERIC 1R (5′-ATG TAA GCT CCT GGG GAT TCA C-3′) and ERIC2 (5′-AAG TAA GTG ACT GGG GTG AGC G-3′) were described previously.[7] Reaction mixtures were subjected to the following thermal cycling parameters in a GeneAmp 9700 thermal Cycler (Applied Biosystems, Warrington, UK): 95°C for 5 min followed by 35 cycles of 95°C for 45 sec, annealing temperature 52°C for 1 min, 72°C for 5 min, followed by a final extension at 72°C for 20 min. Following amplification, PCR products were visualised on 1.5% (w/v) Certified Low Range Ultra Agarose (Bio-Rad Laboratories, Hertfordshire, UK) containing ethidium bromide (1 µg/mL). Gels were analysed by ultraviolet (UV) illumination with a gel image analysis system (UVP Products, Cambridge, UK) and banding profiles were determined manually, where a unique genotype was defined as having one or more differences in its banding profile.

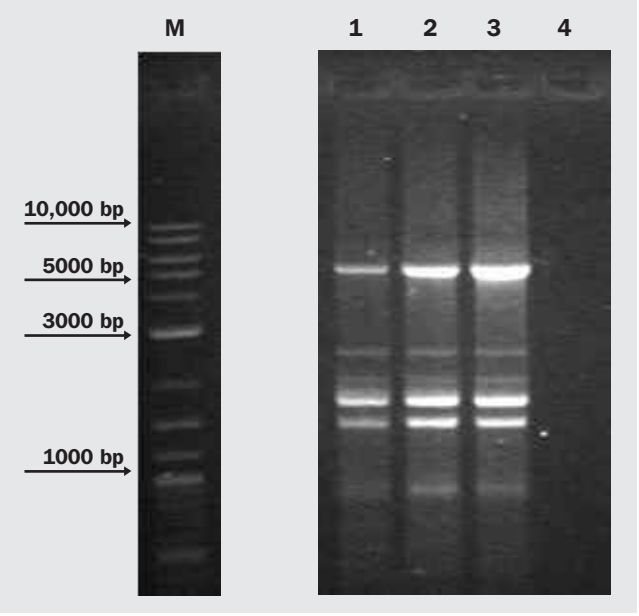

Fig. 1. Comparison of molecular genotypes as represented by ERIC-PCR profiles of *E. coli* O157:H7 NCTC12900, before, during and after X-ray exposure. Lane M: molecular weight marker (New England Biolabs, UK), lane 1: ERIC-PCR DNA profile before X-ray exposure, lane 2: ERIC-PCR DNA profile at the end of X-ray exposure, lane 3: ERIC-PCR DNA profile of first passaged cells following completion of X-ray exposure, lane 4: negative control (molecular grade water).

Results

There was no alteration in antibiotic susceptibility with any of the four bacterial organisms examined. Table 1 details the antibiotic susceptibility of each organism to 11 antibiotic agents and shows the size of zone (mm) of inhibition prior to X-ray exposure, during X-ray exposure and after exposure. Employing the Student's *t*-test, there was no significant

Table 2. Comparison of minimum inhibitory concentrations (MICs) with six antibiotic agents tested against four organisms before, during and after exposure to X-ray radiation.

		Minimum inhibitory concentration					
		Trovafloxacin	Piperacillin	Ceftazidime	Ciprofloxacin	Ceftriaxone	Cefotaxime
Staphylococcus aureus NCTC6571	Before	0.32	0.19	2	0.064	1	0.75
	During	0.32	0.19	2.5	0.064	1	0.75
	After	0.32	0.19	2.5	0.079	1	0.75
Enterococcus faecium	Before	0.19	0.5	3	0.5	0.75	0.125
	During	0.19	0.5	3	0.5	0.75	0.125
	After	0.19	0.5	3	0.5	0.75	0.125
Escherichia coli NCTC12900	Before	0.094	1	0.125	0.008	0.064	0.075
	During	0.125	1	0.125	0.008	0.05	0.064
	After	0.094	1	0.125	0.008	0.047	0.047
Pseudomonas aeruginosa NCTC10662	Before	0.38	1.5	0.5	0.032	3	3
	During	1	1.5	0.5	0.032	4	4
	After	0.38	1.5	0.4	0.032	3	3

Before: analysis of antibiotic susceptibility before X-ray exposure.

During: analysis of antibiotic susceptibility at the end of X-ray exposure.

After: analysis of antibiotic susceptibility on first passaged cells following completion of X-ray exposure.

difference between antibiotic susceptibilities using combined datapoints, either before and during exposure ($P=0.9$) or between before and after exposure ($P=0.77$). Table 2 details the MIC of each organism prior to X-ray exposure, during X-ray exposure and after exposure. Likewise, with these MIC data, there was no significant difference in MIC value between the before and during time points ($P=0.68$), as well as between the before and after time points ($P=0.96$). Regarding alteration to the whole-genome DNA ERIC-PCR profile of each organism, there was no change either during or after exposure, as illustrated in Figure 1.

Discussion

McMahon previously defined environmental stress as "an external factor that has an adverse effect on the physiological welfare of bacterial cells, leading to reduction in growth rate, or in more extreme circumstances, to inhibition and/or cell death, at individual or population levels".[1] Exposure of bacterial cells to clinical X-ray radiation could therefore be considered an environmental stress, as this property has been used to eliminate entire bacterial populations, particularly in food safety/preservation systems.

Several environmental stresses induce the multiple antibiotic resistance (*mar*) operon,[9] which regulates the expression of several genes, including those that encode a broad-specificity efflux pump.[10] In addition, stress hardening[11] may lead to cross-protection against a range of apparently unrelated stress challenges, including resistance to antibiotics. Therefore, bacterial cells have several mechanisms to select for mutants from within sublethally stressed bacterial populations, as well as to minimise stress and maximise continued cell viability to ensure survival following the removal of the stress conditions.

More recently, the term 'stressosome' has been proposed, which describes a signal transduction cascade that increases the expression of stress-response genes and, where stress signals may be integrated by a multiprotein signalling hub, responds to various signals to effect a single outcome.[12]

Bacteria living on a patient's skin, as commensal organisms, such as the coagulase-negative staphylococci (*Staphylococcus epidermidis*) or in the gastrointestinal tract, as well as infecting pathogens, will be exposed to X-ray radiation when the patient undergoes such imaging examination. The potential outcomes of such exposure for the bacteria include i) they remain unharmed, ii) they survive with DNA damage, iii) they survive with radiation-induced genomic instability, iv) they are killed by the radiation. In such circumstances, genetic instability may manifest as mutated genes, which may lead to an altered bacterial genome with important downstream clinical consequences.

The emergence of antibiotic-resistant nosocomial pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter* spp., extended-spectrum β-lactamase (ESBLs)-producing organisms (e.g., *E. coli* and *P. aeruginosa*) has led to the examination of the origins of such resistance. One hypothesis may be that X-ray exposure, with resulting bacterial genetic instability, may lead to *de novo* generation of resistance in clinical organisms and nucleic acid rearrangements within the bacterial genome. Very little is known about the effects of X-ray radiation on commensal and pathogenic organisms presenting on/with patients undergoing radiological examination. Hence, it is necessary to explore any effect that X-ray exposure could have on the bacterial population on such patients.

In conclusion, exposure of these organisms to this amount of X-ray radiation did not alter the organisms' antibiotic susceptibility nor their genomic DNA profiles – these data indicate that exposure of bacteria to X-ray radiation does not lead to mutational events which increase antibiotic resistance. Furthermore, it did not alter genomic DNA profiles and therefore does not compromise molecular epidemiological tracking of bacteria within healthcare environments in which patients have undergone X-ray exposure. □

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