EXPERT REVIEWS

Recent advances in the potential interconnection between antimicrobial resistance to biocides and antibiotics

Expert Rev. Anti Infect. Ther. 11(4), 363–366 (2013)

Marco R Oggioni*[1], Leonardo Furi[1], Joana R Coelho[2], Jean-Yves Maillard[3] and José L Martínez[4]

[1]*LAMMB Dipartimento di Biotecnologie Mediche, Università di Siena, Siena, Italy*
[2]*INESC-ID/IST Technical University of Lisbon, Lisbon, Portugal*
[3]*Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, UK*
[4]*Centro Nacional de Biotecnologia, CNB–CSIC, Madrid, Spain*
**Author for correspondence:*
Tel.: +39 0577 233101
oggioni@unisi.it

Workshop on biocides: do they select for antimicrobial resistance? Second International Conference on Antimicrobial Research

Lisbon, Portugal, 21–23 November 2012.

Interconnection between microbial resistance to biocides and antibiotics is a topic of increasing interest given the recent changes in European legislation and claims of a risk of biocide use on bacterial resistance. In the second International Conference on Antimicrobial Research held in Lisbon in November 2012, a workshop specifically addressed this topic, presentations included approaches to risk assessment and investigations into the molecular mechanisms of biocide resistance and co- and cross-resistance to antibiotics. The overall conclusion was that, even if each biocide represents a specific case, there is scientific evidence that biocides select for biocide resistance, but that there is, so far, no conclusive evidence that this also determined or will determine an increase in antibiotic resistance.

Biocides have been widely used for decades, but despite this widespread and increasing use, most bacterial and fungal species remain susceptible to biocides. However, decreased susceptibility of bacteria and fungi to biocides has been reported and occasionally linked to antibiotic resistance. These reports have raised concern about a risk of increasing antibiotic resistance connected to the use of disinfectants in the environment, hospitals and industry. In view of this growing concern and the new licensing requirements recently posed in Europe, protocols are urgently needed to allow risk assessments for the use of biocidal products. The workshop entitled 'Biocides: do they select for antimicrobial resistance?' opened the second International Conference on Antimicrobial Research (ICAR) [101] held on 21–23 November 2012 in Lisbon. The workshop and the meeting in general provided a strong conceptual and experimental framework within which to answer these questions.

We have to recognize that over the last four decades, biocides have been increasingly used in a number of consumer products, and yet our understanding of bacterial behavior when exposed to these agents is limited. Factors affecting a decrease in bacterial susceptibility to biocides have been described and can be split into those depending upon the biocide (knowledge of the concentration exponent and mechanism of action, i.e., cellular targets, are key), those depending upon the microorganisms (a number of cellular mechanisms conferring an increase in insusceptibility have been reported), and those depending on application, such as exposure parameters and formulation of the biocide [1–3]. Although the potential for bacteria to survive biocide exposure is real, there is a lack of harmonization in the test methodologies used to measure the extent of bacterial resistance or decrease in biocide susceptibility, with protocols used often lacking a realistic approach [1–3]. There is a need for the

 10.1586/ERI.13.16 ISSN 1478-7210

development of predictive methodologies, based on *ex situ* test principle. This would require generating appropriate baseline data from using harmonized protocols and a better understanding of the conditions of use of biocides to set up appropriate test parameters.

Methods for modeling of resistance data and risk assessment approaches were among the first presentations to be discussed at the meeting. In order to find new methodologies useful for the study of impact of biocide use, Joana R Coelho (Technical University of Lisbon, Lisbon, Portugal) proposed the application of different machine learning methods, namely decision trees validated with permutation tests and clustering techniques, to investigate a large dataset of 1600 clinical isolates of *Staphylococcus aureus* [4]. Results showed new associations between antibiotic resistance and reduced susceptibility to biocides, where the standard statistical approaches had failed to deal with the complexity of the data. More precisely, a high MIC for benzalkonium chloride and chlorhexidine, but not of other biocides, was identified as rule for the prediction of multidrug resistance (MDR) in staphylococci.

The molecular analysis of biocide-induced antibiotic resistance was introduced by José L Martínez (Centro Nacional de Biotecnologia-CSIC, Madrid, Spain). The aim of the work was to evaluate which of two possible resistance mechanisms is induced by different classes of biocides: stable resistance due to the acquisition of mutations and transient resistance triggered by the induction of a resistance mechanism in the presence of the biocide. The analyses were performed on the model organism *Stenotrophomonas maltophilia*, an opportunistic pathogen presenting low susceptibility to antibiotics [5,6]. Mutation rates toward resistance to triclosan, benzalkonium chloride and hexachlorophene were measured, and triclosan mutants were found to be resistant to antibiotics due to overexpression of the MDR efflux pump SmeDEF [7]. In selected cases, benzalkonium chloride mutants were also resistant to antibiotics, but none of hexachlorophene mutants presented resistance to antibiotics. All antibiotic resistance mutants presented associated fitness costs. Altogether, this indicates that the probability of selecting antibiotic resistant mutants with biocides ranks triclosan > benzalkonium chloride, without any probability in the case of hexachlorophene. The survival of these mutants might be improved with associated fitness costs. Transient induction of resistance by triclosan was achieved through the binding of the biocide to SmeT [8], the regulator of SmeDEF expression [9], and the consequent overexpression of the efflux pump. However, the observed level of antibiotic resistance was lower than predicted because triclosan concentrations that induce SmeDEF expression are toxic for bacteria.

Molecular mechanisms of biocide resistance and methods for prediction of risk of resistance were investigated in the model organism *S. aureus* by Marco R Oggioni (University of Siena, Siena, Italy). He outlined the different scenarios encountered when analyzing different biocides. Three scenarios were described. In the first scenario, the biocide triclosan was shown to efficiently select for resistance towards itself, but lacked significant overlap of these mutations to resistance genotypes in clinical isolates. Most importantly, triclosan was not associated with an association to antibiotic resistance, both in mutants and clinical isolates [10,11]. In contrast, chlorhexidine and benzalkonium chloride were quite ineffective in the selection of *in vitro* mutants and, in addition, they did not allow the prediction of reduced susceptibility in clinical isolates mainly due to plasmid encoded multidrug efflux pumps. Reduced susceptibility for these two biocides showed a very low correlation coefficient with antibiotic resistance. For the biocide sodium hypochlorite (bleach), no resistance could be selected *in vitro* and no correlation to antibiotic resistance was detected. In summary, as for *S. maltophilia* (see above), in staphylococci, a case-by-case investigation is needed to gain insight into possible risks associated with biocide use. Following this, Leonardo Furi (University of Siena) showed the discovery of a new resistance mechanism to the biocide triclosan in *S. aureus* [10]. The presence of an additional, chromosomally encoded *sh-fabI* allele, horizontally transferred from *Staphylococcus haemolyticus* was documented in about half of the clinical isolates with reduced susceptibility to triclosan. This is the first demonstration that a biocide could exert a selective pressure that is able to drive the spread of a resistance determinant in a human pathogen.

Transcriptomic methodologies have been extensively applied to study triclosan resistance. This is the case of the work of Catherine Burgess (Teagasc Food Research Centre, Ashtown, Ireland) who reported triclosan-induced expression of genes involved in metabolism, transport and chemotaxis, in particular of genes of the flagellar assembly pathway. Putative alternative triclosan targets to *fabI* were described in the poster of Jae-Gu Pan (Korea Research of Bioscience and Biotechnology, Daejeon, Republic of Korea). Among a number of candidate genes, overexpression of *pgsA*, *rcsA* or *gapC* conferred a similar level of triclosan resistance induced by *fabI* overexpression to *Escherichia coli* cells. These results indicated that triclosan may have multiple targets other than well-known *fabI* [12]. Gene expression analysis of triclosan-resistant *S. aureus* clinical isolates and mutant strains was investigated by Denis Grandgirard (University of Bern, Bern, Switzerland). The author confirmed the upregulation of *fabI* in triclosan-resistant clinical isolates. Interestingly, the clinical isolate with the highest level of *fabI* overexpression was characterized by IS256 insertion upstream the transcriptional start site, indicating IS-element-induced transcription as drug-resistant mechanism.

In addition to the work in *Stenotrophomonas*, the substrate specificity of efflux pumps was evaluated in a series of other bacteria and yeasts [13–15]. Emmanuela Marchi and Carlo Viti (University of Firenze, Firenze, Italy) presented a detailed phenotypic characterization of *qacA*, *qacB*, *qacC qacG* and *norA* MDR efflux pumps in *S. aureus*. Thanks to the phenotype microarray high-throughput technology [13], known efflux targets were confirmed and new potential efflux substrates identified. Efflux-mediated biocide resistance mechanisms were also evaluated in *Streptococcus thermophilus* by Stefania Arioli (University of Milan, Milan, Italy), especially focusing on the *pmrB* efflux pump. Quantitative real-time reverse-transcription PCR analysis revealed the induction

of *pmrB* expression when cells were exposed to chlorhexidine or ethidium bromide, and cloning showed association with chlorhexidine, alexidine and the antibiotics tetracycline and furaltadone, suggesting a possible cross-resistance phenotype. Epidemiological linkage between decreased susceptibility to quaternary ammonium compounds, antibiotic resistance and the AcrAB-TolC system in clinical isolates of *E. coli* was described by Sylvie Buffet-Bataillon (Centre Hospitalier Universitaire de Rennes, Hopital Pontchaillou, Rennes, France) [15]. The susceptibility of yeast and mould isolates was presented by Ayse Kalkanci (Gazi University School of Medicine, Ankara, Turkey) who showed correlation of the *CDR2* MDR efflux pumps and triclosan resistance. The study presented, to our knowledge, is one of the first applied to yeast isolates.

In addition to susceptibility tests in microdilution, data on biofilms were also presented. Lucilla Baldassarri (Istituto Superiore di Sanità, Roma, Italy) pointed out that a direct correlation between ability to form biofilm and resistance to benzalkonium chloride was not detectable in staphylococci. This report and the work of Hana Turonova (Institute of Chemical Technology in Prague, Prague, Czech Republic) on *Listeria monocytogenes* both confirmed the well-described increased resistance of biofilms to biocides, when compared with planktonic organisms [16].

In addition to the above presentations, the ICAR meeting gave a much wider overview on biocides, especially nonantibiotic biocides. Still the scope of this article is to report the the work of the experts present at the ICAR meeting. During the initial workshop on 'Biocides: do they select for antimicrobial resistance?', extensive discussion developed between the speakers and the audience on the significance of data available for risk evaluation. All main speakers agreed that overuse or misuse of biocides may present a risk for selection of biocide resistance and that it would be advisable to attentively monitor the use of biocides based on the 'precaution principle'. Conversely, all speakers also agreed that so far, the extension of the correlation between biocide use and reduced biocide susceptibility cannot be extended toward clinically relevant antibiotic resistance at present. Instrumental to this point was the large number of presentations of the FP7 funded BIOHYPO project [102], which had analyzed the reduced susceptibility to biocide and possible correlation to antibiotic resistance *in vitro* and in clinical isolates. So far, data from the BIOHYPO project do not allow for the indication of any risk of clinically significant antibiotic resistance development following the use of biocides. Further work will be needed as every biocidal compound or product has distinct biological mechanisms of action and resistance, and the effects have so far been investigated on only a few model species.

Financial & competing interests disclosure

Scientists participating in the BIOHYPO project, funded by the European Commission (Contract 227258), provided help and advice in the organization of the workshop. JY Maillard's expenses to attend and present at International Conference on Antimicrobial Research were paid by The Society of Applied Microbiology. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

1 Cottell A, Denyer SP, Hanlon GW, Ochs D, Maillard JY. Triclosan-tolerant bacteria: changes in susceptibility to antibiotics. *J. Hosp. Infect.* 72(1), 71–76 (2009).

2 Maillard JY, Hartemann P. Silver as an antimicrobial: facts and gaps in knowledge. *Crit. Rev. Microbiol.* doi:10.3109/1040841X.2012.713323 (2012) (Epub ahead of print).

3 Maillard JY. Bacterial resistance to biocides in the healthcare environment: should it be of genuine concern? *J. Hosp. Infect.* 65(Suppl. 2), 60–72 (2007).

4 Coelho JR, Carriço JA, Knight D *et al*. The use of machine learning methodologies to analyse antibiotic and biocide susceptibility in *Staphylococcus aureus*. *PLoS ONE* 8(2), e55582 (2013).

5 Sanchez MB, Hernandez A, Martinez JL. *Stenotrophomonas maltophilia* drug resistance. *Future Microbiol.* 4(6), 655–660 (2009).

6 Sánchez MB, Martínez JL. SmQnr contributes to intrinsic resistance to quinolones in *Stenotrophomonas maltophilia*. *Antimicrob. Agents Chemother.* 54(1), 580–581 (2010).

7 Sanchez P, Moreno E, Martinez JL. The biocide triclosan selects *Stenotrophomonas maltophilia* mutants that overproduce the SmeDEF multidrug efflux pump. *Antimicrob. Agents Chemother.* 49(2), 781–782 (2005).

8 Hernández A, Ruiz FM, Romero A, Martínez JL. The binding of triclosan to SmeT, the repressor of the multidrug efflux pump SmeDEF, induces antibiotic resistance in *Stenotrophomonas maltophilia*. *PLoS Pathog.* 7(6), e1002103 (2011).

9 Sánchez P, Alonso A, Martinez JL. Cloning and characterization of SmeT, a repressor of the *Stenotrophomonas maltophilia* multidrug efflux pump SmeDEF. *Antimicrob. Agents Chemother.* 46(11), 3386–3393 (2002).

10 Ciusa ML, Furi L, Knight D *et al*. A novel resistance mechanism to triclosan which suggests horizontal gene transfer and demonstrates a potential selective pressure for reduced biocide susceptibility in clinical strains of *Staphylococcus aureus*. *Int. J. Antimicrob. Agents* 40(3), 210–220 (2012).

11 Oggioni MR, Ciusa ML, Furi L *et al*. Lack of evidence for reduced fitness of clinical *Staphylococcus aureus* isolates with reduced susceptibility to triclosan. *Antimicrob. Agents Chemother.* 56(11), 6068–6069; author reply 6072 (2012).

12 Yu BJ, Kim JA, Ju HM *et al*. Genome-wide enrichment screening reveals multiple targets and resistance genes for triclosan in *Escherichia coli*. *J. Microbiol.* 50(5), 785–791 (2012).

13 Decorosi F, Santopolo L, Mora D, Viti C, Giovannetti L. The improvement of a phenotype microarray protocol for the chemical sensitivity analysis of *Streptococcus thermophilus*. *J. Microbiol. Methods* 86(2), 258–261 (2011).

14 Tocci N, Iannelli F, Bidossi A *et al*. Functional analysis of pneumococcal drug efflux pumps associates the MATE DinF transporter with quinolone susceptibility. *Antimicrob. Agents Chemother.* 57(1), 248–253 (2013).

15 Buffet-Bataillon S, Le Jeune A, Le Gall-David S, Bonnaure-Mallet M, Jolivet-Gougeon A. Molecular mechanisms of higher MICs of antibiotics and quaternary ammonium compounds for *Escherichia coli* isolated from bacteraemia. *J. Antimicrob. Chemother.* 67(12), 2837–2842 (2012).

16 Purkrtova S, Turonova H, Pilchova T, Demnerova K, Pazlarova J. Resistance of *Listeria monocytogenes* biofilms to disinfectants. *Czech J. Food Sci.* 28(4), 326–332 (2010).

Websites

101 Second International Conference on Antimicrobial Research. www.formatex.org/icar2012

102 BIOHYPO. Confronting the clinical relevance of biocide induced antibiotic resistance. http://kdbio.inesc-id.pt/biohypo