Disinfectant Rotation – A Microbiologist's View

An examination of the ongoing discussion of the validity of rotation of disinfectants in a pharmaceutical cleanroom

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I HAVE MODERATED THE PMFLIST, an email discussion list on the Internet dedicated to topics of interest to microbiologists in the pharmaceutical and personal care industries; a list that has been active since 1996 (http://www.microbiol.org/pmflist_info.htm). I mention this only because in that time I have had the opportunity to develop a perspective on issues that interest the working microbiologist. Occasionally, the odd topic will really catch fire in the discussion group. This happened recently in response to an interesting article that appeared in the March 2005 issue of *A2C2 Magazine* [1] dealing with the advisability of disinfectant rotation. In this thoughtful article, the author argued for the need to "rotate" a disinfectant with a sporicide. The interest in this topic initially came as a surprise to me, as I had thought the issue long since resolved in the pharmaceutical and personal care industries.

As the discussion continued on the PMFList, the points of contention became more clear. A great deal of discussion revolved around the concept that a microorganism could become resistant to a disinfectant. Here is where the first bit of clarification is required. A microorganism will not become resistant to much of anything. It either is or it is not affected by the compound. Within a very large population of microorganisms, there is a chance (normally a chance of approximately 10^{-6}) that a cell within the population will have a mutation at a specific gene. This might provide some competitive advantage under some environments, perhaps survival in the presence of an elevated level of a chemical. The mutation that promotes survival under these conditions could, over the course of a few generations, become the dominant genotype in that population. This is, of course, assuming that the challenge is not too great. For example, it does not matter how acidotolerant a particular mutant is, growth in 6N hydrocholoric acid is just plain unlikely—the conditions are too inhospitable to life.

Can a low level of exposure to biocides select variants in a population that are more tolerant than the previous dominant type? Can low level exposure select "resistant" microorganisms? Absolutely. The phenomenon of biocide resistance is well known and has been extensively reviewed in the literature [2-5]. Extensive work on the mechanism of action allowing previously susceptible microorganisms to survive elevated levels of the biocide have highlighted efflux pumps as a major contributor to the biocide resistance [6,7], as well as physiological adaptation [8].

The next question is one of significance. Is this phenomenon of any consequence to the pharmaceutical cleanroom sanitization program? Well, that is what I hope to answer in this short essay.

The Development of Resistance in Bacterial Populations

Genetic Resistance

The literature provides many examples of microorganisms able to survive in disinfectants. This can be either in laboratory experiments using an increasing level of biocide to select variants in the population, or by examination of biocidal solutions for the presence of resistant microorganisms. The gram-negative bacilli are the most frequent isolates from this type of evaluation [9-15]. This may be due to a combination of causes including alterations in outer membrane permeability due to changes in porin diameters [16-19]. In addition, it is not clear that the outer-membrane mediated resistance is in fact due to selection of a mutant genotype from the population, or rather phenotypic adaptation, as this trait has been reported to be rapidly lost once the selective pressure is removed.

The other type of experiment performed to demonstrate development of genetic resistance is one in which a laboratory researcher takes a microorganism in culture and exposes it to increasing levels of biocides, selecting resistance variants at each stage. There is one such set of experiments of particular relevance to the pharmaceutical microbiologist that we will examine in some detail as an example.

In 1992, Conner and Eckman published a study purporting to show the need for rotation of a low pH phenolic and a high pH phenolic to prevent the generation of resistant *Pseudomonas aeruginosa* [20]. This study was a repetitive zone of inhibition design, where an alkaline (pH 10.4) and an acidic (pH 2.6) phenolic disinfectant were placed in paper discs at the center of a bacterial lawn. As the lawn grew to visible turbidity, the disinfectant diffusing out from the disc created a concentration gradient. Conner and Eckman then picked colonies that grew closest to the disc, therefore in the highest concentration of disinfect in the diffusion gradient, for the next cycle. Four treatments were used: water; high pH phenolic; low pH phenolic; and alternating (rotating) the two phenolics. The challenge was over 40 cycles of picking a "resistant" colony, creating a lawn using this new isolate, and then looking for another "resistant" colony from the first variant.

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What the authors found was that the alkaline phenolic treatment eventually resulted in a variant of *P. aeruginosa* that produced no zone of inhibition around the alkaline phenolic-impregnated disc, while the low pH phenolic and the "rotated" phenolics had measurable zones at the end of 40 cycles. One plausible explanation for these results is that the alkaline phenolic is a poor disinfectant. However, Conner and Eckman concluded that these data demonstrated the need for rotating disinfectants. They provided no explanation for the fact that the low pH phenolic seemed to be as effective as the "rotated" phenolics at preventing "resistance" from developing.

They continued this work in a further study published in 1993 [21] and 1994 [22] where an adherent *P. aeruginosa* biofilm was formed on a stainless steel coupon and then subjected to repeated cycles of disinfection (by dipping the coupon in the use dilution) and reinoculation. Survivors were determined by sampling with replicate organism detection and counting (RODAC) plates. The authors showed that no treatment eliminated the established biofilm, but the rotation provided a statistically significant decrease in the numbers of colony forming unit (CFU) than in either the high or the low pH treatment. Unfortunately, the difficulty with using biofilms in this type of study is that the biofilm itself can adapt to different environments [23, 24]. This well-studied phenomenon confounds interpretation of the data. In addition, there was no attempt by the authors to determine the sampling efficacy of RODAC plates on biofilm generated under the various conditions. Given the well-documented inefficiency of this sampling method [25, 26] and the variations in biofilm structure [27-31] interpretation of the study becomes a bit more difficult.

Unfortunately, there is a dearth of other articles in the literature demonstrating the need to rotate disinfectants. Given what we know about population variability, the infrequent rate of favorable genetic mutations, and the mechanism of action of many of these biocides, it seems that the probable scenario for selection of

a resistant variant would require exposure of an extremely large number of cells (in excess of 1,000,000 CFU) to a low level of the toxic chemical. It is not surprising that this has not been reported in the literature from a pharmaceutical manufacturing cleanroom facility. In fact, this has not been reported even for hospital situations where both the levels of microorganisms are much higher and the potential for recognition of the event is more likely [32, 33]. Put simply, selection of mutants that are resistant to in-use levels of disinfectants has not been shown to happen in cleanroom settings. Literature reports of resistance to in-use levels are restricted to descriptions of the survival of specific microorganisms in contaminated solutions [9, 14, 34].

Physiological Adaptation

While we have discussed genetic adaptation, there is another mechanism for resistance. In the previous section we touched on biofilms. A biofilm is a complex community of microorganisms suspended in a polysaccharide glycocalyx. The extracellular structure provides a foundation, nutrients for some members of the community, and also physical protection from chemical treatment as it impedes the diffusion of chemicals to the cells in the interior. The ability of biofilm to withstand large levels of disinfectants is well established [35-37]. This does not, however, speak to the need to rotate disinfectants, as the biofilm will provide protection against whichever chemical treatment you attempt, as shown earlier by Conner and Eckman.

There is one other aspect of this discussion I should mention. In the aforementioned article [1], the author correctly separated the disinfection of vegetative cells from the need to provide sporicidal activity to eliminate spores of bacteria and fungi. Although there are clear differences among different bacterial species in their sensitivities to disinfectants and these differences can result in persistent bacterial load under some conditions, these differences pale beside the resistance of spores to environmental insult. The spore form is naturally more resistant to chemical treatments and harsher agents must be used to combat these organisms. This is the basis of the common industrial practice of alternating the daily use of a disinfectant with the periodic use of a sporicide in a manufacturing facility's sanitization program. The obvious approach to this problem might seem to be the exclusive use of the sporicide. However, as the author points out, the

consistent use of common sporicides (frequently, strong oxidizing agents) will result in corrosion of equipment in a relatively short period of time and pose safety issues for the technicians applying them to the cleanroom. It is far preferable to use the gentler disinfectant for as much of the time as possible, reserving the sporicide for periodic cleaning, response to an event, e.g. power failure, catastrophic excess of action levels, or bringing a facility back on-line after a shut-down.

On the Need for Clarity in Terms

A final aspect of this discussion is the semantics of the subject matter. A real problem is the use of the term "resistance." This term originated in the clinical microbiology arena where the inhibition of bacterial growth provides a true measure of the efficacy of a particular chemical agent against that bacterial species. This measure has little meaning in disinfectancy where the true test is the ability of the agent to kill bacteria, not prevent them from growing. So we start off with the wrong test. From this poor beginning we then argue that a slight but measurable increase in the ability of the organism to grow in low levels of the chemical agent is proof of resistance, ignoring the fact that the dilution of the agent in a facility may be thousands of times more concentrated than the concentration used in the study [38]. Finally we ignore the basic mechanism of action of disinfectants, incorrectly applying the model of antibiotic resistance (where a mutant develops resistance to a "magic bullet" by altering the specific target of the antibiotic) to the mode of action of disinfectants (most of which act at a basic chemistry level: completely disrupting the cell membrane or fundamentally altering entire classes of cell components). A resistance that can be measured only in the lab, not in the field, is of little practical concern.

A second problem is the term used to describe the pharmaceutical practice. Given our current understanding, describing what we do as "disinfectant rotation" is grossly inaccurate. We, in fact, are not discussing rotating disinfectants at all. Rather we are urging the routine use of an effective disinfectant with the periodic use of a sporicide [39, 40]. Block [41] defines a disinfectant as, "...an agent that frees from infection, ...that destroys disease or other harmful microorganisms but may not kill bacterial spores. It refers to substances applied to inanimate objects." He goes on to define a sporicide as "...an agent that destroys microbial spores, especially a chemical substance that kills bacterial spores."

What we are discussing is a practice more accurately described as a sanitization program, but certainly not "disinfectant rotation"—a term that continues to confuse practitioners and regulators alike.

Summary

The need for the rotation of disinfectants in a pharmaceutical cleanroom sanitization program is not supportable from a scientific basis. The assumptions that proponents of the practice assert as facts, e.g. generation of resistant organisms, greater efficacy of alternating agents, are not supported by the literature. However, even when using a validated disinfectant as part of a well-managed cleanroom sanitization program, periodic use of a sporicide is a prudent—even an essential—component of the sanitization program. It is needed to address the occasional appearance of spore-forming organisms in the environmental monitoring program and therefore ensure the cleanest possible environment for manufacturing. We need to clearly describe our practices and leave behind the inaccurate

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phrase "disinfectant rotation," as it does not describe the current practice of an effective cleanroom sanitization program and only confuses discussion of the issues involved.

References

1. E.K. Sartain, "Regulatory Update: Rotating Disinfectants in Cleanrooms: Avoid Going in Circles," A2C2 Magazine, Vol. 8, No. 3, (March, 2005) pp. 32-33.
2. A.D. Russell. "Mechanisms of Resistance to Antiseptics, Disinfectants and Preservatives," Pharmacy Int., Vol. 7, (1986) pp. 305-308.
3. K. Poole. "Mechanisms of Bacterial Biocide and Antibiotic Resistance," J. Appl. Microbiol. Sympos. Suppl., Vol. 92, (2002) pp. 5S-64S.
4. D.G. White, P.F. McDermott. "Biocides, Drug Resistance and Microbial Evolution. Current Opinion in Microbiology," Vol. 4, (2001) pp. 313-317.
5. A.D. Russell, et al. "Microbial Susceptibility and Resistance to Biocides," ASM News, Vol. 9, (1997) pp. 481-487.
6. S.B. Levy, et al. "Active Efflux, a Common Mechanism for Biocide and Antibiotic Resistance," J. Appl. Microbiol. Sympos. Suppl., Vol. 92, (2002) pp. 65S-71S.
7. D. Nies. "Efflux Mediated Heavy Metal Resistance in Prokaryotes," FEMS. Vol. 27, (2003) pp. 313-319.
8. X. Liu, et al. "Global Adaptations Resulting from High Population Densities in *Escherichia coli* Cultures," J. Bacteriol. Vol. 182, No. 15, (2002) pp. 4158-4164.
9. G.A.J. Ayliffe, et al. "Contamination of Disinfectants," Brit. Med. J., Vol 1, (1969) pp. 505-511.
10. S.G. Geftic, et al. "Fourteen-Year Survival of *Pseudomonas cepacia* in a Salts Solution Preserved with Benzalkonium Chloride," Appl. Environ. Microbiol., Vol. 37, No. 3, (1979) pp. 505-510.
11. R. Berkleman. "*Pseudobacteremia* Attributed to Contamination of Povidone-Iodine with *Pseudomonas cepacia*," Annals of Internal Medicine, (1981) pp. 32-36.
12. T.J.W.C. Marrie. "Prolonged Survival of Serratis marcescens In Chlorhexidine," J. Appl. Environ. Microbiol., Vol. 42, No. 6, (1981) pp. 1093-1102.
13. R.L. Sautter, et al. "*Serratia marcescens* Meningitis Associated with a Contaminated Benzalkonium Chloride Solution," Infect. Control," Vol.5, (1984) pp. 223-225.
14. R.L. Anderson, et al. "Investigations into the Survival of *Pseudomonas aeruginosa* in poloxamer-Iodine," Appl. Environ. Microbiol., Vol 47, No. 4, (1984) pp. 757-762.
15. R.L. Anderson, et al. "Prolonged Survival of a Strain of *Pseudomonas cepacia* in a Commercially Manufactured Povidone-Iodine," Appl. Environ. Microbiol., Vol. 56, (1990) p. 3598.
16. R.E.W. Hancock. "Alterations In Outer Membrane Permeability," Ann. Rev. Microbiol., Vol. 38, (1984) pp. 237-264.
17. H. Nikaido, M. Vaara. "Molecular Basis of Outer Membrane Permeability," Microbiol. Rev., Vol. 49, (1985) pp. 1-32.
18. H. Nikaido. "Outer Membrane Barrier As a Mechanism of Antimicrobial Resistance," Antimicrob. Agents Chemother., Vol. 33, No. 11, (1989) 1831-1836.
19. R.L. Anderson. "Investigations of Intrinsic *Pseudomonas cepacia* Contamination in Commercially Manufactured Povidone-Iodine," Infect. Control Hosp. Epidemiol., Vol. 12, No. 5, (1991) pp. 297-302.
20. D.E. Conner, M.K. Eckman. "Rotation of Phenolic Disinfectants," Pharm. Technol. (September, 1992) pp. 148-160.
21. D.E. Conner, M.K. Eckman. "Rotation of Phenolic Disinfectants Enhances Efficacy Against Adherent *Pseudomonas aeruginosa*," Pharm. Technol., Vol. 6, No. 1, (1993) pp. 94-104.
22. D.E. Conner, M.K. Eckman. "Rotation Of Phenolic Disinfectants Enhances Efficacy

Against Adherent *Pseudomonas aeruginosa*," Pharm. Tech. Eur., Vol. 6, No. 1, (1994) pp. 44-47.
23 K. Lewis. "Riddle of Biofilm Resistance," Antimicrob. Agents Chemother., Vol. 45, No. 4, (2001) pp. 999-1007.
24 S. Rachid, et al. "Effect of Subinhibitory Antibiotic Concentrations on Polysaccharide Intercellular Adhesin Expression in Biofilm-Forming *Staphylococcus epidermidis*," Antimicrob. Agents Chemother., Vol. 44, No. 12, (2000) pp. 3357-3363.
25 M.S. Favero, et al. "Microbiological Sampling of Surfaces," J. Appl. Bacteriol., Vol. 31, (1968) pp. 336-343.
26 W. Whyte. "Methods for Calculating the Efficiency of Bacterial Surface Sampling Techniques," J. Hosp. Infect., Vol. 13, (1989) pp. 33-41.
27 P. Stoodley, et al. "Growth and Detachment of Cell Clusters from Mature Mixed-Species Biofilms," Appl. Environ. Microbiol., Vol. 67, No. 12, (2001) pp. 5608-5613.
28 T. Tolker-Nielsen, et al. "Development and Dynamics of *Pseudomonas sp.* Biofilms," J. Bacteriol., Vol. 182, No. 22, (2000) pp. 6482-6489.
29 K. Sauer, et al. "*Pseudomonas aeruginosa* Displays Multiple Phenotypes during Development as a Biofilm," J. Bacteriol., Vol. 184, No. 4, (2002) pp. 1140-1154.
30 K. Sauer, et al. "Characterization of Nutrient-Induced Dispersion in *Pseudomonas aeruginosa* PAO1 Biofilm," J. Bacteriol., Vol. 186, No. 21, (2004) pp. 7312-7326.
31 V.E. Wagner, et al. "Microarray Analysis of *Pseudomonas aeruginosa* Quorum-Sensing Regulons: Effects of Growth Phase and Environment," J. Bacteriol., Vol. 185, No. 7, (2003) pp. 2080-2095.
32 M.T.E. Suller, A.D. Russell. "Antibiotic and biocide resistance in methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*," J. Hosp. Infect., Vol. 43, (1999) pp. 281–291.
33 M.T. Suller, RA.D. Russell, "Triclosan and antibiotic resistance in *Staphylococcus aureus*," J. Antimicrob. Chemother., Vol. 46, (2000) pp. 11–18.
34 S. Langsrud, et al. "Characterization of *Serratia marcescens* Surviving in Disinfecting Footbaths," J. Appl. Microbiol., Vol. 95, (2003) pp. 186-195.
35 D.J. Stickler. "Susceptibility of Antibiotic-Resistant Gram-Negative Bacteria to Biocides: a Perspective from the Study of Catheter Biofilms," J. Appl. Microbiol. Sympos. Suppl., Vol. 92, (2002) pp. 163-170S.
36 C. Ntsama-Essomba. "Resistance of *Escherichia coli* Growing As Biofilms to Disinfectants," Vet. Res., (1997) pp 353-363.
37 P. Gilbert, et al. "Influence of Growth Rate On Susceptibility to Antimicrobial Agents: Biofilms, Cell Cycle, Dormancy and Stringent Response," Antimicrob. Agents Chemother., Vol. 34, No. 10, (1990) pp. 1865-1868.
38 P. Gilbert, et al. "Biocide Abuse and Antimicrobial Resistance: Being Clear About the Issues," J. Antimicrob. Chemother., Vol. 50, (2002) pp. 137-148.
39 USP. <1072>, "Disinfectants and Antiseptics," Pharm. Forum. , Vol. 30, No. 6, (2004) pp. 2108-2118.
40 USP. <1116>,"Microbiological Control and Monitoring Environments used for the Manufacture of Healthcare Products," Pharm. Forum., Vol. 31, No. 2, (2005) pp. 524-549.
41 S.S. Block. "Definition of Terms," Disinfection, Sterilization and Preservation 4th Ed. S.S. Block, ed. (Philadelphia, Lea & Febiger, 1991) pp. 18-25.

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