results, the authors conclude that there may be transmission of MRSA between animals and humans. If this conclusion is correct, it begs the question of whether the transmitted MRSA strains are human-associated strains. If the transmission is a livestock-associated strain, is it a human health concern? Further research is needed to address these important questions.

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Herman J. Gibb, PhD, MPH*
Gibb Epidemiology Consulting LLC
Arlington, VA

Keri G. O'Leary, MS
Gibb Epidemiology Consulting LLC
Arlington, VA

Milken Institute School of Public Health
George Washington University
Washington, DC

Zsuzsanna Jeney, MPH
Gibb Epidemiology Consulting LLC
Arlington, VA

*Address correspondence to Herman J. Gibb, PhD, MPH, Gibb Epidemiology Consulting LLC, 3033 Wilson Blvd, Ste 700, Alexandria, VA 22201.
E-mail address: Herman.Gibb@gibbepi.com (H.J. Gibb).

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Biofilm removal: Erroneous methodologies cause even more confusion?

To the Editor:

We would like to bring the readers' attention to the gross experimental error in the article "Evaluation of detergents and contact time on biofilm removal from flexible endoscopes" by Ren et al[1] that completely invalidates the results and conclusions of the article.

It appears the authors overlooked our warning[2] on a similar results-invalidating error made by Vickery et al[3] in 2004 that before using protocols based on enumerating bacterial survivors, the absence of cytotoxicity of the tested detergent must be confirmed. It is simply wrong to equate the killing of bacteria to the removal of the biofilm from the surface.

The minimum bactericidal concentration (MBC) of benzalkonium chloride (BAC) on *Escherichia coli* is approximately 45 ppm.[4] The Intercept detergent (tested by Ren et al[1]) contains 4.8% BAC,[5] or 480 ppm, at use dilution of 1:100. In other words, the authors disregarded the presence of interfering substance at levels of approximately 10 times greater than the MBC. Our previous letter[2] was pointing at the same experimental error when Vickery et al[3] were assaying biofilms by enumerating survivors after exposure of the biofilm to 1,000 ppm of BAC (>20 times greater than the MBC). To confirm, we performed a quick test on the MBC of BAC against *E coli* (as per the U.S. Environmental Protection Agency [EPA] methodology[6]) and confirmed that there were no survivors when approximately 200 organisms of *E coli* (ACCC 8196) were exposed to 100 ppm of BAC (B6295 Sigma) for 2 minutes. It is of no surprise that both groups of researchers have not recovered any survivors after exposing biofilms to the interfering substance at concentrations of 10-20 times the MBC. All AOAC, European Standard, and U.S. EPA test protocols emphasize the need for cytotoxicity validation, and it is rather surprising that the authors overlooked this textbook validation step.

Strong cationic detergents such as BAC (used in both Matrix and Intercept formulations; Sigma Aldrich, St. Louis, MO) result in dense clusters of dead cells as can be seen in the Scanning Electron Micrographs images from Ren et al[1] (Fig 1A and Fig 1B). These clusters form pockets of protein and carbohydrate-rich bioburden, which potentially interferes with the subsequent disinfection-sterilization step. The thicker the bioburden, the greater the probability of failure. If one evaluate the distribution of bioburden using the U.S. EPA criteria for biofilm cleaners—"prepares the surface for application of a registered disinfectant intended to kill biofilm,"[7]—the surface covered by easily accessible single cells as in Fig 1B[1] is far more desirable than the dense clustered nutrient-rich conglomerates of Fig 1A.[1] In other words, when using the U.S. EPA criteria, the rating of the cleaners and conclusion of the study should be exactly opposite to the one made by the authors.

The authors[1] have also chosen to deviate significantly from standard methods,[8] with respect to replacing the nutrient-poor

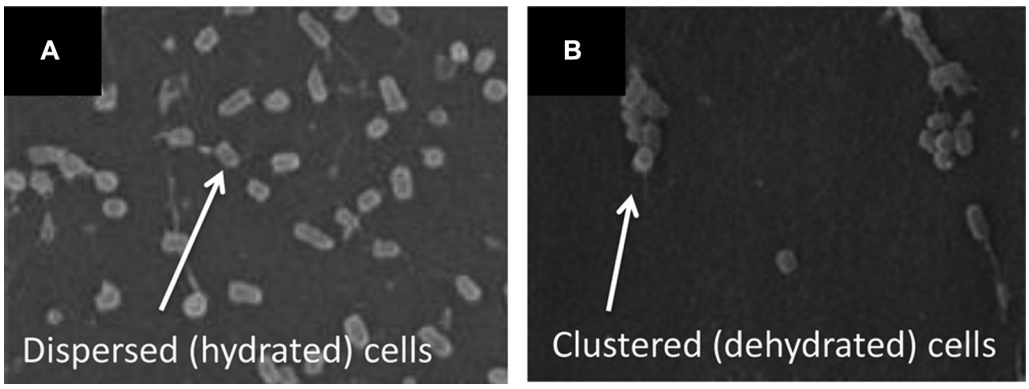

Fig 1. Scanning Electron Micrograph micrographs taken from Ren et al[1] indicating (A) disperse and hydrated cells and (B) clustered and dehydrated dead cell bodies.

conditions for nutrient-rich conditions and replacing the validated recommended test organism (biofilm forming strain of *Pseudomonas aeruginosa*) for *E coli*. Both deviations appear rather unwarranted: it is widely accepted that the biofilm in endoscope lumens is most likely to occur during storage when airborne *P aeruginosa* is contaminating washed and disinfected (ie, nutrient poor) lumen. The scenario envisaged by the authors—biofilm resulting from *E coli* from patient feces—is extremely unlikely.

The biofilm removal and accessibility of bacteria to disinfectant action should be regarded as a major risk when assessing the feasibility of replacing manual brushing with automated reprocessing in automatic endoscope reprocessors. The research on removal of biofilms is expensive, time consuming, and limited to specialized laboratories only. This is why the wider infection control community heavily relies on the published results—the conclusions of the articles similar to Ren et al[1] and Vickery et al[3] are copied-and-pasted into process risk assessment reports and product marketing sheets. The erroneous test methodologies result in erroneous conclusions that in turn lead to underestimating the risks and might cause major outbreaks, similar to the recent incident at University of California, Los Angeles.[9]

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Alex Sava, PhD
Damian Kirchmajer, PhD*
Novapharm Research Australia
Rosebery, NSW, Australia

* Address correspondence to Damian Kirchmajer, PhD, Novapharm Research (Australia) Pty Ltd, 3-11 Primrose Ave, Rosebery, NSW 2018, Australia.
E-mail address: damian.kirchmajer@regionalhealth.com.au (D. Kirchmajer).

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Effect of geographic region and seasonality on *Clostridium difficile* incidence and hospital mortality

To the Editor:

The recent study by Argamany et al[1] concluded that the incidence and hospital mortality for *Clostridium difficile* infection (CDI) differed between major regions of the United States and across different seasonal times of the year. However, these conclusions were not supported by the data in their study because the authors based them exclusively on statistical significance without considering the effect size of their findings. The effect sizes of region and season on CDI were very low or near zero, contradicting their conclusion, as subsequently explained.

The effect sizes for U.S. region (Northeast, Midwest, South, and West) and seasonality (winter, spring, summer, and fall) were estimated using the data for patients overall and presented in their Figures 1-5.[1] The population rates provided were first converted into a contingency table with population counts from which Pearson χ^2 was calculated. The χ^2 was converted to a Pearson correlation coefficient, which is a standard estimator of effect size[2] using the conversion formula of $r = \chi^2 / (\chi^2 + N)$, for when the degrees of freedom are >1. The effect sizes were interpreted using Cohen's recommended criteria.[3]

The reanalysis of data showed that effect sizes (r) for the effect of U.S. region on CDI incidence was $r = 0.016$, and the effect of season was $r = 0.003$. The effect of region on CDI hospital mortality was $r = 0.014$, and the effect of season was $r = 0.023$. By Cohen's criteria,[3] an effect size of $r = 0.10$ is considered a small effect size, but these effect sizes average less than a tenth of that value, approaching zero. Although statistically significant because of the enormous sample size in the study ($N = 2{,}279{,}004$), the differences of the CDI incidence and patient mortality, as the effect sizes estimates indicated, are so trivial that the regional and seasonal differences can be safely ignored. This is consistent with the small percentage differences reported in the study: the CDI mortality for all patients differed only by approximately 1% between regions or seasons, and incidence differed across seasons by a fraction of a percent.

An additional complication is that there was little agreement on the riskiest region and season. For overall CDI mortality, the highest risk seasons were fall for adults and winter for older adults. On the other hand, for CDI incidence, the riskiest seasons were spring for both adults and older adults. Therefore, there was no agreement between incidence and mortality measures for CDI, which leaves open the question of whether any single season can be identified as the highest risk season. The same is true regarding regional differences, with the Northeast having the highest risk for CDI incidence, but the Midwest region having the worst for hospital mortality. The lowest risk was the Northeast for mortality and West for incidence. Therefore, similar to seasonality, no single region can really be identified as the high-risk region overall.

To sum up, the conclusion that the CDI incidence and hospital mortality of patients significantly differed among regions and seasons is contradicted by the calculated effect sizes and other inconsistencies in the findings. Our additional analysis does not support the authors' suggestion that "These results underscore the need for improved infection control and antimicrobial stewardship measures to prevent CDI and its transmission, particularly in high-risk regions and seasons." Instead, our estimates that the effect size