Antimicrobial activity of silver doped fabrics for the production of hospital uniforms

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SUMMARY

Among several alternatives to control hospital-acquired infections (HAIs), a strategy could be the use of hospital uniforms imbued with antimicrobial substances. For this purpose we evaluated the antibacterial activity of two different silver doped fabrics employed for the production of hospital uniforms. The study was conducted in two-step. In the first the antimicrobial activity was evaluated *in vitro* against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212. In the second, we tested the total viable counts detected from beginning to end of the work shift on experimental silver doped uniforms worn by doctors, nurses, allied health assistants in different hospital wards. The *in vitro* tests showed a remarkable antibacterial activity of both silver doped samples (>99.9% reduction within 4h of exposure for Gram-positive and within 24h for Gram-negative bacteria). The experimental uniforms provided results only slightly in agreement with *in vitro* data. Even if the increase of total viable counts was somewhat lower for experimental uniforms than traditional ones, significant differences were not observed. Despite the results on the uniforms worn, the addition of silver in fabrics to make medical equipment (supplies) remains an interesting option for HAI control.

KEY WORDS: Silver, Antibacterial activity, Fabrics, HAIs, Hospital uniforms

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INTRODUCTION

A health care–associated infection (HAI) is a localized or systemic condition that results from adverse reactions to the presence of an infectious agent(s) or its toxin(s) and that is not present or incubating at the time of admission to the hospital (Horan et al., 2008). A HAI usually breaks out in hospitalized patients, but also it could affect hospital staff. The problem of HAI is directly related to different causes: a microbiological hazard that could be present in workplaces or the hospital environment; care, diagnostic or therapeutic procedures; concomitant diseases. In general, HAIs affect any part of the human body and are often compounded by a complicated therapy due to multidrug-resistant strains circulating in hospitals. Despite progress in public health and hospital care, and in spite of the reduction in the duration of hospital stays and in the number of hospitalized patients, the frequency of HAIs has not decreased in the past 20 years worldwide causing a significant impact both in terms of human lives and cost savings (5% of patients admitted to hospital contract an infection in hospital) (Wenzel and Edmond, 2001; Klevens et al., 2002; Rosenthal et al., 2002; Scott, 2009). In the U.S. it is estimated that approximately 100,000 patients die every year from HAI-related causes (Klevens et al., 2002), with an estimated annual cost up to $33 billion (Scott, 2009). In European countries from 5% to 20% of hospitalized patients (between 450,000 and 700,000) contract nosocomial infections leading to death in 10% of cases with an annual cost up to € 15.5 million (Lizioli et al., 2000; Nicastri et al., 2003; Di Pietrantonj et al., 2004; Pellizzer et al., 2008).

Among the different alternatives to control HAIs, in addition to good hygiene practices, an effective prevention tool could be the use of medical supplies that can limit the spread of microorganisms. One possibility could be to use antimicrobial fabrics for the production of hospital linen and clothing (gowns, uniforms, sheets, pillowcases), that play a crucial role in

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the chain of infection being suitable to carrying bacteria and acting as a reservoir for their transmission (Tinker, 2010; Singh et al., 2012). It may thus be useful to adopt silver doped fabrics to produce hospital uniforms, whose ideal characteristics must be long-lasting antibacterial properties, a broad spectrum of antimicrobial activity, an effect limited to surface tissue and an absence of toxicity to humans. Silver ions are well known to be effective against a broad range of microorganisms and are used to control bacterial growth in a variety of medical applications (Jung et al., 2008). Silver ions attack multiple sites within the cell to inactivate critical physiological functions (cell wall synthesis, membrane transport, nucleic acid synthesis and translation, protein folding and function, and electron transport) without which the microorganism is inhibited from growth, loses its infectivity or is killed (Bragg and Rainnie, 1974; Richards et al., 1984; Thurman and Gerba, 1989; Furr et al., 1994; Gibbins and Warner, 2005). The activity depends on both the concentration of silver ions and the sensitivity of the microbial species to silver (Dibrov et al., 2002; Mirjalili et al., 2013). Contact time, temperature, pH and presence of free water all affect both the rate and extent of antimicrobial activity although it was observed that when treated with silver Gram-negative bacteria are subject to more structural damags than Gram-positive organisms (Feng et al., 2000; Cooper, 2004). These differences could be explained based on the structure of the cell wall. Gram-positive bacteria have more peptidoglycan than Gram-negative bacteria because of their thicker cell walls, and because peptidoglycan is negatively charged and silver ions are positively charged, more silver may get trapped (Kawahara et al., 2000; Pal et al., 2007).

The lower susceptibility of Gram-positive bacteria can also be explained by the fact that their cell wall is thicker than that of Gram-negative bacteria and then it is more difficult to penetrate.

In view of the above considerations, in collaboration with Siggi Group S.p.A. (VI, Italy), a manufacturer of professional clothes including hospital uniforms, we have evaluated the

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antimicrobial activity of fabrics containing silver. Initially, the test was carried out on silver doped fabric artificially contaminated with pathogenic/opportunistic bacteria frequently responsible for hospital-acquired infections. Subsequently, the antibacterial activity was determined on uniforms made with the same textiles worn by health workers in different wards of Modena University Hospital.

MATERIALS AND METHODS

Bacterial strains

Four reference microorganisms, belonging to the species most frequently associated with HAIs (Marion Grare et al., 2007) were employed: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212. All cultures were maintained on Tryptic Soy agar (TSA, Oxoid, Mi, Italy) slants and sub-cultured monthly. Each strain was cultured overnight in Tryptic Soy broth (TSB, Oxoid, Mi, Italy) at 37 °C, harvested by centrifugation at 10,000g for 10 min at 4 °C and rinsed twice with sterile saline solution (0.9% NaCl). The rinsed bacteria were resuspended in saline solution to obtain about 10^6 CFU/ml, and the viable counts determined on TSA agar plates. A 100-µl aliquot containing about 10^5 Colony Forming Units (CFU) of each of the strains was used as a cell suspension.

Test materials

Two different fabrics were tested for their antimicrobial activity, a sample 180 (a fabric with a plain weave, the most basic of three fundamental types textile weaves) and a sample 215 (a fabric with a twill 2/1, a type of textile weave with a pattern of diagonal parallel ribs). Both samples have a composition 50% polyester / 50% cotton and were packed with silver ions on a ceramic carrier, incorporated in the polyester fibers. The antimicrobial activity was also

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evaluated on samples previously subjected to 30 and 50 washing cycles (180 W30 and 180 W50; 215 W30 and 215 W50) using as negative control the undoped fabrics treated to the same washing cycles. The washing cycle was: pre washing 10 min 35-60°C with non-ionic and anionic surfactants; washing 10 min 75 °C non-ionic and anionic surfactants and hydrogen peroxide; rinsing one 3 min 20 °C; rinsing two 3 min 20°C; rinsing three 3 min 20°C; neutralization 4 min 20 °C with peracetic acid.

In vitro *antimicrobial activity evaluation of the silver doped fabric samples*

Autoclaved samples, cut into squares of 2 x 2 cm, were inoculated, from time to time, with a 100-µl aliquot of suspension (about 10^5 CFU) of *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 6538 or *E. faecalis* ATCC 2912 and their antibacterial activity was evaluated at different exposure times (1, 2, 4, 8, 12, 24 hours). Fabrics without antibacterial substances were used as controls. The artificially contaminated textile samples were maintained in glass jars under a humidified atmosphere (steadily greater than 70% relative humidity) and incubated at 30 °C. At the established times, each fabric sample was put into 10 ml of sterile saline solution, and, after vigorous shaking with vortex mixer for 5 min, appropriate dilutions (100 µl) were spread on TSA. If no growth was observed, the bacterial count was determined by filtration (0.45 mm-pore-size filter; Millipore Corp.) of the entire volume of the suspension.

The measure of the antimicrobial activity expressed as the arithmetic mean of the percentage reduction (R) was calculated with the following mathematical formula:

$R = (B-A) / (B) \times 100$

R: percentage reduction of the microbial cells

A: CFU of the sample microbial suspension after 1, 2, 4, 8, 12, 24 h

B: CFU of the control microbial suspension after 1, 2, 4, 8, 12, 24 h

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All experiments were repeated three times. The means were plotted against incubation time and the standard deviation was reported as error bars. The rates of decline of the indicator strains were analyzed with a t-test for paired data. Statistical probability equal to or less than 0.05 was considered significant.

Antimicrobial activity evaluation of the silver doped uniforms worn

In a study design on uniforms worn, the contamination of the traditional uniforms (TUs) was compared after a work shift with that observed in the experimental uniforms (EUs) made with 180 silver doped fabric. The uniforms were worn by doctors (Ds), nurses (Ns) and allied health assistants (AHAs) belonging to three different wards of Modena University Hospital: pediatrics 88 uniforms (46 EUs and 42 TUs), surgery 93 uniforms (43 EUs and 50 TUs) and long-term care unit 62 uniforms (EUs 37 and 25 TU). The different number of uniforms worn for the experiment was due to hospital staff availability. Evaluation of the antimicrobial activity was carried out comparing the number of CFU recovered at the beginning (t_0) and at the end (t_1) of the work shift, by contact plate method (55 mm petri dish, TSA, Oxoid). For each uniform six samplings were performed (three at the beginning and three at the end of the work shift) choosing as contact points three areas frequently in contact with hands and at risk of contamination: right pocket, left pocket and small pocket.

All plates were incubated at 37 °C and, after 48 h, the CFU of each uniform was calculated as the sum of colonies growth on the three plates. In order to obtain a single value for each uniform, the ratio (t_0 / t_1) between the CFU at the beginning and at the end of the work shift was calculated. To obtain more information about the microorganisms found on uniforms, those belonging to Micrococcaceae, Enterococcaceae, Enterobacteriaceae and Pseudomonadaceae families were isolated by replica plating on selective media. For this purpose, for each uniform, two plates obtained respectively at the beginning and at the end of

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the work shift from the same contact point, were replicated on McConkey agar, Cetrimide agar, Kanamycin-Aesculin-Azide agar and Mannitol Salt agar (all from Oxoid). After incubation, the microorganisms were preliminarily identified by colony morphology, Gram stain, catalase and oxidase testing and, in some cases of doubt, confirmed by biochemical systems (BBL Enterotube II and Oxi/Ferm Tube II, Becton Dickinson Diagnostic System, Pont de Claix, France; API 20 strep and API staph, bioMérieux, Marcy-l'Etoile, France).

RESULTS

In vitro *antimicrobial activity evaluation of the silver doped fabric samples*

Tables 1-2 and Figure 1(a, b, c, d) display the results concerning the *in vitro* antimicrobial activity evaluation of the textile samples. Both silver doped samples (180 and 215) showed a remarkable activity compared to fabrics without antibacterial substances (control). The reduction of Gram-negative bacteria (*E. coli* and *P. aeruginosa*), still detectable after the first hour of contact, exceeded 90% at the second hour, while at the same hour ranging for Gram-positive bacteria from 40% (*E. faecalis*) to 75% (*S. aureus*). After the 4^{th} hour we observed a 99% reduction for *E. coli*, 98% for *P. aeruginosa* and *S. aureus* while *E. faecalis* (57%) confirmed a lower susceptibility in agreement with Mariscal *et al*. (2011). Starting from the 8^{th} hour and up to the end of the experiment, the reduction was more than 99.9% for Gram-negative while for Gram-positive, especially *E. faecalis*, this result was achieved only at the 24^{th} hour.

The antibacterial activity of the washed fabric samples (180 W30 and 180 W50; 215 W30 and 215 W50) against the Gram-negative bacteria was generally lower in the first hours compared to not washed samples, but at the end of the experiment reached the same value. An unexpected result was the significant reduction (>99%) of *S. aureus* and in particular of *E. faecalis* even after 2 hours especially considering the lower susceptibility of this

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microorganism towards not washed samples. In all cases, at the end of the experiment, the differences in antibacterial activity between control and silver doped samples, analyzed with a t-test for paired data, were highly significant ($p<0.001$).

Antimicrobial activity evaluation of the silver doped uniforms worn

The antimicrobial activity of the silver doped uniforms worn was evaluated as the mean values of the ratio t_0 / t_1 of CFU determined at the beginning (t_0) and at the end (t_1) of the work shift for both traditional (TUs) and experimental uniforms (EUs). Though the colony forming units to the end of the work shift increased for both types of uniforms, generally the increases were smaller for the EUs. For the pediatric ward, the mean value of the ratio t_0 / t_1 was equal to 0.72 for EUs and 0.58 for TUs; for the long-term care unit the mean value of the ratio t_0 / t_1 was equal to 0.77 for EUs and 0.57 for TUs; for the surgery ward the mean value of the ratio t_0 / t_1 was equal to 0.46 for EUs and 0.49 for TUs. The latter value did not show a behavior consistent with the *in vitro* tests.

Evaluating the data in more detail, 52% of the EUs tested in the Pediatric ward showed, between t_0 and t_1, an increase in total viable counts lower than 50% (value $t_0 / t_1 > 0.5$); the remaining 48% presented a greater increase (value $t_0 / t_1 < 0.5$). Among the TUs, in 55% of cases there was an increase in total viable counts greater than 50% (value $t_0 / t_1 < 0.5$) and only the remaining 45% a value $t_0 / t_1 > 0.5$. Among the EUs tested in the long-term care unit, 67% showed a value $t_0 / t_1 > 0.5$ while the traditional uniforms in 60% of cases only. Among the EUs tested in the surgery ward, 34% showed a value $t_0 / t_1 > 0.5$ while the TUs in 36% of cases. No significant differences among the number of colony forming units detected in the uniforms worn by doctors, nurses and allied health assistants belonging to three different wards were observed.

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Among the bacteria isolated and identified, Gram-positive organisms (Micrococcaceae and Enterococcaceae families) were more represented and no important difference in their number was found comparing the TUs and EUs from the beginning and the end of the work shift. A dissimilar trend was observed for the Gram-negative bacteria (Enterobacteriaceae and Pseudomonadaceae families) because their number decreased at the end of the work shift in the EUs only. This greater susceptibility of the Gram-negative bacteria to the silver doped textiles is consistent with the *in vitro* results.

DISCUSSION

Despite improved hygiene and infection control programs, the transmission of bacteria to and from patients remains of great concern. In terms of transmission, fabrics play a crucial role in the chain of infection for pathogenic/opportunistic microorganisms (Tinker et al., 2010; Singh et al., 2011). Therefore, hospital staff uniforms endowed with antimicrobial properties may be of great help in reducing the occurrence and spread of hospital-acquired infections. A number of biocidal treatments to give antimicrobial properties to fabrics and bring hygienic or performance benefits are now available (Singh et al., 2011) and in this context the use of silver is an interesting solution. In particular, silver nanoparticles are of great interest because of their easy production, high antimicrobial activity, low toxicity to humans and capability to be incorporated into different types of products. For this purpose we evaluated the antimicrobial activity of fabrics containing silver to be used in the hospital uniform production. In the *in vitro* study the silver doped fabrics showed a remarkable antibacterial activity, with a better activity against Gram-negative bacteria, especially *E. coli*. The washed samples, on the contrary, were more active against Gram-positive microorganisms, a finding that needs further study. The evaluation of antibacterial activity performed on experimental

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uniforms worn by healthcare workers unexpectedly provided results not always in agreement with the data obtained *in vitro*. Even if the increase in the total viable counts from beginning to end of the work shift was slightly lower for experimental uniforms than traditional uniforms, the difference was not significant. A similar result was reported by Groß *et al*. (2010) who compared the contamination rates of newly developed silver-hybrid clothing worn by ambulance personnel during one week of emergency medical service with that of standard textile clothing. Groß *et al*. (2010) showed that surprisingly the concentration of the bacteria on the clothing incorporating silver threads, not only does not decrease, but it was higher than on the conventional clothing, especially after the third working day.

It is not easy to understand why on the silver doped uniforms worn we have not shown the remarkable antibacterial activity obtained from the *in vitro* tests. Many variables can influence the survival capacity of microorganisms make it difficult to understand more deeply the contribution of contaminated inert materials to their transmission (Mariscal et al., 2011). An essential condition is the environmental humidity degree and the temperature (Michels *et al*., 2009). While our *in vitro* test was carried out at 30°C with a humidity steadily greater than 70%, measured with a hygrometer, in the test on the uniforms worn the temperature and the humidity degree were not always constant and generally much lower; probably the silver doped uniforms for these reasons were unable to show the expected antimicrobial activity.

Although the results of our study on the uniforms worn were not entirely encouraging, the use of silver as antimicrobial agent remains an exciting possibility especially in the medical field. It remains to be understood in what hospital context the silver fabrics can be applied in order to fully express their antibacterial activity. For example, a pilot study with 30 patients (Gabbay *et a*l., 2006) showed a statistically significantly lower colonization rate in patients sleeping in beds using biocidal sheets compared with those who slept on regular sheets. Therefore, also considering the silver low toxicity to humans, a possible application could be

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in the sheets and pillowcases, etc., i.e. in fabrics subjected to a greater degree of humidity being more intimately in contact with patients.

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355 **Table 1.** Gram negative bacteria: percentage (%) of reduction

E. coli ATCC 8739						
Samples	**1 h**	**2 h**	**4 h**	**8 h**	**12 h**	**24 h**
180	95.70	99.58	99.95	99.99	99.99	99.99
180 W30	/	45.00	79.30	96.65	99.80	99.99
180 W50	/	47.46	78.00	98.33	99.70	99.99
215	95.60	96.45	99.78	99.97	99.99	99.99
215 W30	/	44.22	86.16	99.14	99.99	99.99
215 W50	/	47.65	77.79	97.50	98.49	99.99
P. aeruginosa ATCC 27853						
180	91.79	95.80	98.62	99.99	99.99	99.99
180 W30	/	98.10	98.30	98.00	99.62	99.99
180 W50	/	97.00	97.20	97.00	99.00	99.90
215	97.70	98.80	98.46	99.99	99.99	99.99
215 W30	/	97.20	98.30	99.00	99.00	99.99
215 W50	/	96.00	96.70	98.60	99.00	99.99

356 The results are rounded to two decimal places

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Table 2. Gram positive bacteria: percentage (%) of reduction

S. aureus ATCC 6538						
Samples	**1 h**	**2 h**	**4 h**	**8 h**	**12 h**	**24 h**
180	28.60	75.30	98.80	98.70	99.60	99.99
180 W30	/	99.20	99.99	99.99	99.99	99.99
180 W50	/	99.99	99.99	99.99	99.99	99.99
215	40.86	72.57	96.75	96.65	98.70	99.99
215 W30	/	99.99	99.99	99.99	99.99	99.99
215 W50	/	99.75	99.99	99.99	99.99	99.99
E. faecalis ATCC 29212						
180	34.23	40.73	56.68	86.36	87.37	99.85
180 W30	/	99.99	99.99	99.99	99.99	99.99
180 W50	/	99.99	99.99	99.99	99.99	99.99
215	30.10	54.45	57.78	89.45	96.03	99.86
215 W30	/	99.80	99.99	99.99	99.99	99.99
215 W50	/	99.99	99.99	99.99	99.99	99.99

The results are rounded to two decimal places

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362 **FIGURE 1 (a, b, c, d). (a)** Trend of *E. coli* ATCC 8739 viable counts evaluated at different
363 times on silver doped and undoped (control) fabric samples. **(b)** Trend of *P. aeruginosa*
364 ATCC 27853 viable counts evaluated at different times on silver doped and undoped (control)
365 fabric samples. **(c)** Trend of *S. aureus* ATCC 6538 viable counts evaluated at different times
366 on silver doped and undoped (control) fabric samples. **(d)** Trend of *E. faecalis* ATCC 29212
367 viable counts evaluated at different times on silver doped and undoped (control) fabric
368 samples. Undoped control (○), sample 180 (♦); sample 180 W30 (■), sample 180 W50 (▲),
369 sample 215 (□), sample 215 W30 (x), sample 215 W50 (△)

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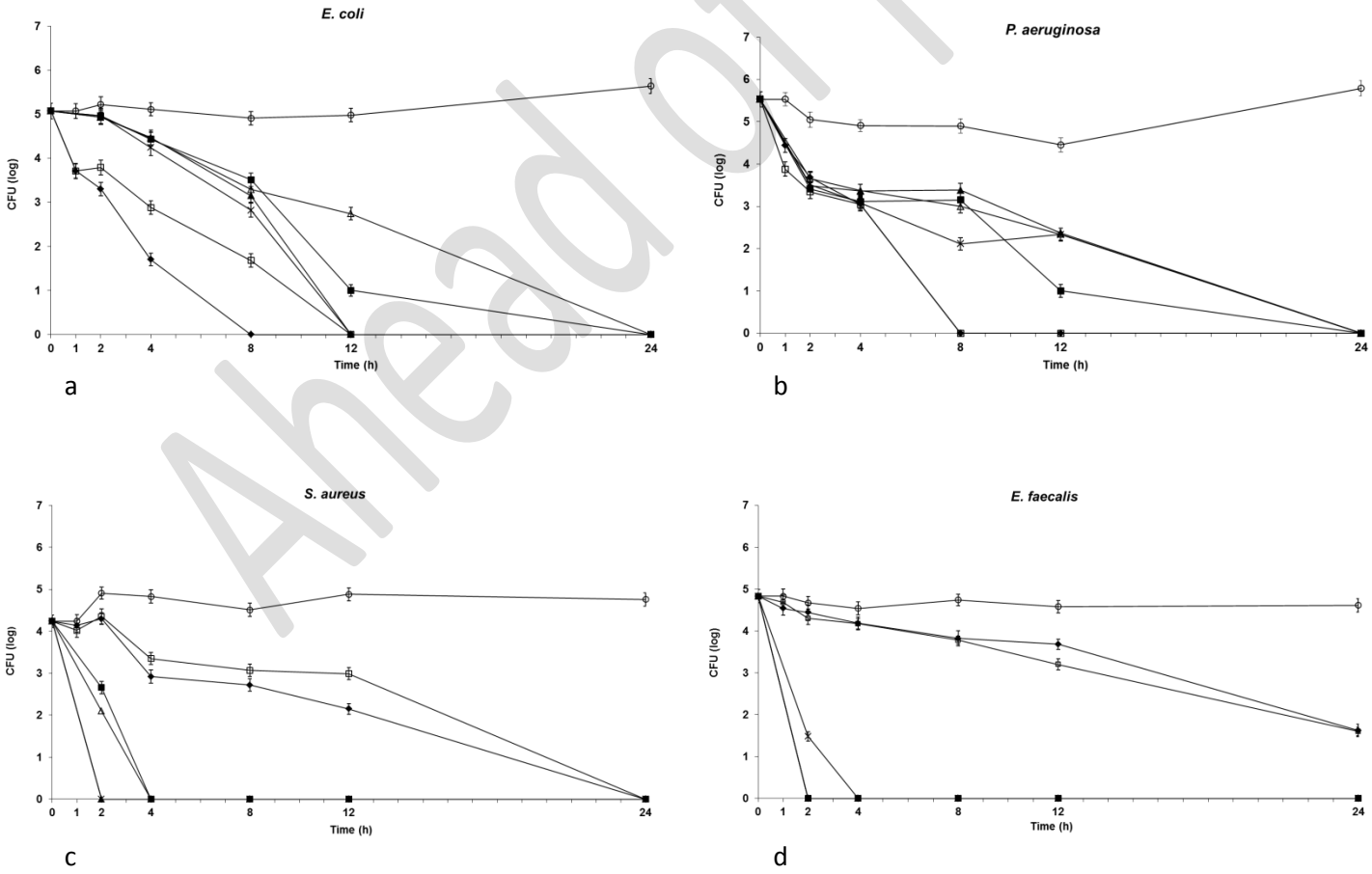

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