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Comparative Study of Surgical Instruments from Sterile-Service Departments for Presence of Residual Gram-Negative Endotoxin and Proteinaceous Deposits[▽]

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The ineffective cleaning of surgical instruments may be a vector for the transmission of hospital-acquired infections. The aim of this research was to investigate whether further decontamination procedures need to be instigated in sterile-service departments (SSDs) to reduce the risk of nosocomial illnesses, such as endotoxemia, sepsis, or iatrogenic Creutzfeldt-Jakob disease (to date, 1,147 cases of confirmed Creutzfeldt-Jakob disease deaths in the United Kingdom since 1990 have been reported). Instrument sets were obtained from nine anonymous United Kingdom National Health Service (NHS) primary care trust SSDs. The investigation implemented an advanced light microscopy technique, episcopic differential interference contrast microscopy with the sensitive fluorescent reagents SYPRO Ruby and 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI), to detect proteinaceous and microbial contamination levels. Gram-negative lipopolysaccharide (LPS) endotoxin was monitored using a dansylated polymyxin B fluorochrome agent. None of the 260 instruments examined displayed signs of microbial colonization or LPS endotoxin contamination. However, over 60 percent of the instruments showed a high degree of protein soiling (0.4 to 4.2 μg protein/mm^2). Some instruments appeared soiled with crystalline deposits that may consist of a potentially hazardous material contributing to inflammation and/or surgical shock. It is clear that the overall standard for cleaning must be raised in order to fulfill the imminent introduction of new European standards and to reduce the risk of cross-patient contamination and iatrogenic transmission.

It is estimated that 15% to 30% of hospital-acquired infections can be prevented through more-effective application of existing knowledge (16, 22). However, it is reportedly difficult to calculate the impact that an improvement in decontamination methods would have (2), although it is well known that failures of conventional procedures have resulted in a wide range of infections (40).

In studies of patients admitted to a general hospital, 17.6% displayed bacteremic episodes, with the most prevalent being caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter*, and *Salmonella* (34). These gram-negative bacteria have lipopolysaccharide molecules or endotoxin on their cell surface, which has been associated with systemic inflammatory infections, such as sepsis. The endotoxin is released from the cell surface of bacteria either through its growth and cell division (small amounts) or on the cell's death (large quantities). These endotoxins are extremely heat stable, remaining viable even after conventional autoclaving (10), and have been shown to require a temperature of 180°C for at least 3 h or 250°C for 30 min to be destroyed (31).

The association between gram-negative bacterial endotoxin and sepsis has been recognized for many years (21), with a large proportion (79%) of sepsis patients also exhibiting endotoxemia (20). Sepsis is a very complicated syndrome that is defined as the invasion of normally sterile tissue, fluid, or body cavity by pathogenic or potentially pathogenic microorganisms (18). Approximately 40% of those with sepsis will progress to septic shock (18), which is the leading cause of morbidity and mortality among hospitalized patients (15).

Unlike commercial providers of decontamination services, who are required to produce evidence that the highest standards of decontamination are met (under Directive 93/42/EEC), the United Kingdom National Health Service (NHS) trusts, which reprocess only their own instruments, are not so required and thus under no obligation to provide any such proof. However, from 2007 onward, the standards set out within the directive (93/42/EEC) will be applied by the United Kingdom Department of Health (Department of Health) to all NHS reprocessing trusts (23). As this deadline becomes closer, it is clear that the need to ensure that high standards are met becomes greater.

In 1999, a "snapshot" survey of the decontamination services within the NHS found instances where decontamination processes did not meet current standards (25). Subsequently, in January 2001, the Department of Health announced that the British Government had allocated £200 million for the improvement of decontamination services and facilities (sterile-service departments [SSDs]) within the NHS by 2003.

The current requirements for the verification and validation of SSD washer/disinfectors (WDs) in the United Kingdom are laid out in Health Technical Memorandum (HTM) 2030 (26). Part of the requirement is that periodic cleaning efficiency tests be performed using the recommended ninhydrin protein detection test to ensure that "residual soil" has been removed (9), although doubts over the test's suitability for detecting low levels of protein residue, including prions, have been raised

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TABLE 1. Defined parameters and equivalent protein concentrations for the contamination index[a]

Contamination index	Particulate ht (μm)	Particulate width (μm)	FOV[b] coverage (%)	Amt of protein per mm^2
1	0–5	0–3	1–2	0–42 ng
2	2–10	3–10	5–10	42–420 ng
3	5–20	10–50	20–50	0.42–4.2 μg
4	20–100	>50	>50	>4.2 μg
4a	20–100	>50	>50	>4.2 μg[c]

[a] Contamination index was defined by amount of protein per mm^2 and calculated based on information whereby a 1-μm-diameter area of protein with an average molecular mass of 30 kDa and of 3 μm in height was calculated to be approximately equivalent to 1 pg (data not shown).

[b] FOV, microscope field of view (0.36 mm^2).

[c] This value was obtained for protein-equivalent soil that did not stain with SYPRO Ruby.

(33). This test is a complicated and time-consuming procedure which has been shown to have a sensitivity (5) of approximately 3 ng/mm^2. However, other detection methods are permitted, including those based on the Biuret reaction (11). The Biuret reaction, which is a simpler procedure, has been reported to display a sensitivity similar to that of the ninhydrin test (4); however, in the presence of lipids and phospholipids, turbidity problems can arise (6). There is no such requirement for testing for endotoxins remaining upon surgical instruments.

Consequently, we have taken advantage of new developments in light microscopy, utilizing episcopic differential interference contrast/epifluorescence (EDIC/EF) techniques (14) for rapid, noncontact examination of even highly curved or serrated surgical instruments, coupled with the use of sensitive fluorescent dyes; SYPRO Ruby (35) is used for the detection of very low levels of protein (17), DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) for the assessment of microbial colonization (3), and dansyl polymyxin B for detecting the presence of endotoxin (32) on "sterile" surgical instrument surfaces.

This report describes an evaluation of the cleanliness of NHS surgical instruments included with instrument trays taken from nine anonymous NHS trusts employing routine detergent or enzymatic cleansers in their WDs.

MATERIALS AND METHODS

Staining. The instruments were assessed for protein contamination using the previously described (17) SYPRO Ruby (Invitrogen) method. In addition to this, the instruments were counterstained with 0.1% (wt/vol) aqueous DAPI (Sigma) solution for 15 min to detect microorganisms. The instruments were incubated in 2.5 μM dansyl polymyxin B (Molecular Probes) for 10 min before being rinsed in endotoxin-free distilled water to detect residual endotoxin.

The stained instruments were visualized using an EDIC/EF microscope under fluorescent illumination with DAPI or dansyl polymyxin B (excitation, 340 to 380 nm; emission, 420 nm [long-pass filter]) or SYPRO Ruby (excitation, 400 to 440 nm; emission, 470 nm [long-pass filter]).

Surgical instruments. Nine surgical instrument sets were received from the Department of Health, and all identification marks had been removed before delivery. The nine sets consisted of over 350 individual instruments, with an average of 40 instruments per set. The instruments were identified by type and size; all instruments found in quantities of one or two per type were tested, but in cases where the instruments were found in quantities of more than two per type, a representative selection (>50%) of that instrument type was examined. In total, 260 instruments were assessed for the presence of residual contamination. All had passed through traditional machine washer-disinfector cleaning procedures and had been deemed clean.

All of the instruments were examined at multiple sample points over their surfaces and scored by applying a contamination index (CI) (17) of between 0 and 4 (Table 1), with 4a being gross contamination but not of a proteinaceous nature, i.e., deposits were readily observable using EDIC microscopy but did not stain with SYPRO Ruby; these contaminants could have included salts, detergent, or enzyme residues from the automated washers.

The defined sample areas of instruments were assessed and scored by comparing the visualized contamination with previously obtained representative images for known contamination indexes. This enabled the rapid assessment of the degree of contamination apparent for each region of interest, and multiregional sampling was performed on all instruments.

The sets were analyzed and subdivided into instrument classes (i.e., hinged or simple). Hinged instruments were defined as those instruments which possess a box joint, e.g., artery forceps (Fig. 1a), while simple instruments were those without a box joint e.g., tongue plates or British Pharmacopeia scalpel handles (Fig. 1b). This comparison was termed intraset.

The instruments were also divided in accordance to type (intertype). Hinged items were investigated more closely due to the identified increased risk of contamination retention within the box joint (19). Accordingly, these instruments were divided into four types, as follows. (i) Tissue forceps (n = 21) are designed to grasp so that the tissues experience minimum trauma during the surgery. (ii) Hemostats (n = 28) are forceps used in surgery to control hemorrhage by clamping or constricting blood vessels. (iii) Towel clips (n = 13) secure drapes to the patient's skin and may be used for holding the tissue as well. (iv) Scissors (n = 17) are used for cutting or dissecting. Finally, (v) needle holders (n = 11) are used to guide needles through tissue during suturing.

Statistical analysis was performed using Kruskal-Wallis analysis of variance on ranks (KW) and the subsequent application of a pairwise multiple comparison procedure (Dunn's method) or by the Mann-Whitney U test. Differences between groups were considered significantly different at P values of <0.05. All statistical analysis was performed using SigmaStat 3.1 (Systat Software Ltd).

RESULTS

The instruments examined had a wide variation in both size and complexity. None of the instruments displayed signs of

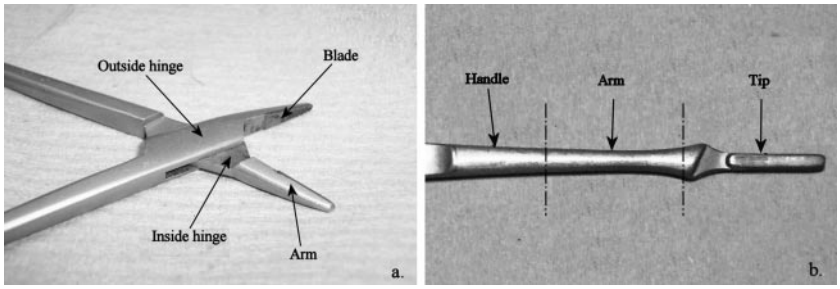

FIG. 1. Examples of the sample regions used for hinged instruments, e.g., Spencer-Wells forceps (a) and simple instruments, e.g., British Pharmacopeia scalpel handles (b).

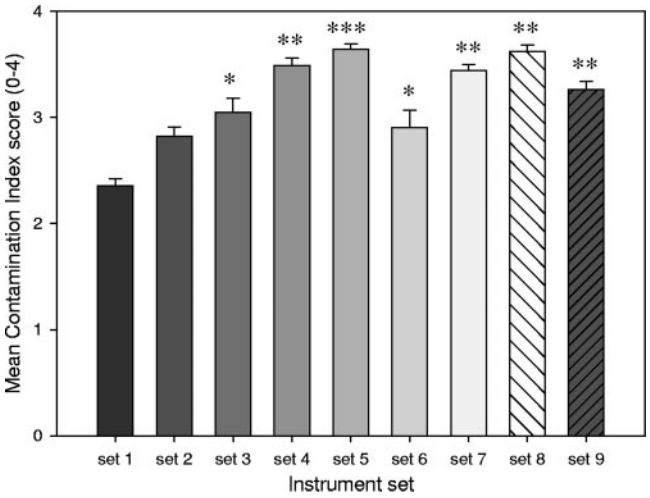

FIG. 2. Mean contamination index scores for the different instrument sets obtained from the nine anonymous NHS trusts. *, significant difference between contamination levels for the instrument set and set 1; **, significant difference between contamination levels for the instrument set and set 1 and 2; ***, significant difference between contamination levels for the instrument set and sets 1, 2, 3, and 6.

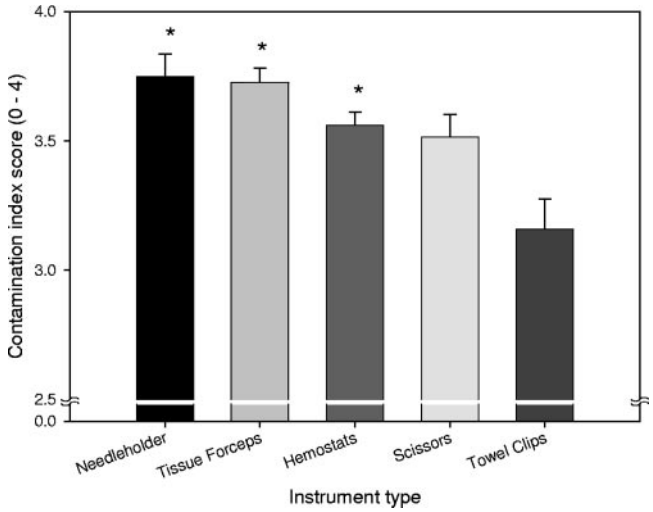

FIG. 3. Comparison of contamination index data obtained from the different types of instrument. Hinged instruments included towel clips (n = 15), tissue forceps (n = 25), hemostats (n = 37), scissors (n = 21), and needle holders (n = 13). *, significant difference between contamination levels for the instrument type and the towel clips ($P < 0.05$).

either microbial contamination or endotoxin soiling, visualized with either DAPI or dansyl polymyxin B, respectively.

Although the degrees and intensities of proteinaceous contamination differed and the protein deposits were not characterized, it was clear that all instruments examined showed signs of proteinaceous contamination on at least one of the sample regions. A previously defined contamination index (17) for protein contamination was implemented to assess the extent of this soiling (Table 1).

The scores were averaged for each instrument; the results indicated that 66% of all the instruments inspected showed severe (CI score, >3 to 4) contamination in at least one of the sample regions, 27% were moderately contaminated (CI score, >2 to 3), and only 7% displayed low-level soiling (CI score, 0 to 2).

Interset relationships. The average contamination index per instrument set differed among the nine trays (range, 2.4 to 3.6), with the overall mean contamination index value for all the instruments being 3.2 (Fig. 2).

Statistical analysis (KW) indicated that there was significant difference in the levels of contamination between the different instrument sets, suggesting that the cleaning procedures in some SSDs are significantly better than those for others.

Intertype. Statistical analysis of the hinged subpopulation showed that there was no significant difference in the levels of contamination between the hinged instruments for all the sets except for tray 1, which was significantly cleaner; as such, the hinged instruments from set 1 were removed from the subsequent analysis (Fig. 3).

Statistical analysis (KW) indicated that there was significant difference in the levels of contamination between the different types of instrument, with the towel clips showing contamination levels significantly lower than those of the other instruments (Fig. 3).

Some instruments displayed areas of crystalline deposition (Fig. 4). These deposits may have been caused by detergent or enzymatic cleaning chemistry residue remaining on the instrument after the rinsing cycle. Indeed, image analysis of photomicrographs obtained for the EDIC and EF channels showed that protein residues were retained more readily on regions of crystalline deposits than on adjacent bare stainless steel surfaces.

DISCUSSION

It is estimated that there are over 2 million cases of hospital-acquired infections in the United States each year, and these incidents are thought to cause around 88,000 suspected deaths per annum (8). This figure creates a substantial socioeconomic burden for the health service, with the extra costs incurred in the United States considered to be in excess of $5 billion (8). Although a large number of these cases, approximately 30%, are thought to be preventable (30), the requirement to produce clean instruments is an "essential prerequisite" for ensuring effective disinfectant or sterilant activity (28).

There are over 6.5 million operations a year performed in England alone (12). These procedures produce approximately 9.2 million (24) surgical trays that require decontamination. With an average of 12 instruments per set (27), this means that approximately 110 million instruments require decontamination per annum, or in real terms, 2 million instruments per week spread over the 249 hospitals with sterile-service departments in England and Wales (25).

The emergence of evidence that highly robust infectious agents, such as the prion protein, a characteristic of variant and sporadic Creutzfeldt-Jakob disease, and septic shock-related endotoxin, may remain viable following standard hospital decontaminating procedures (1, 7, 31, 36, 37) led the Department of Health to issue revised guidelines on the decontamination of instruments (HSC 178_1999 and 179_1999) in August 1999 (38, 39). However, it is clear that subsequent and ongoing

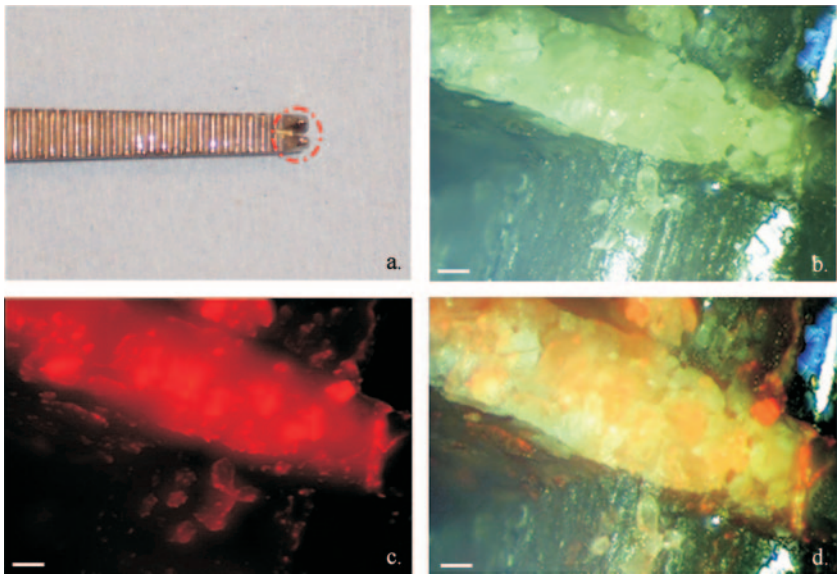

FIG. 4. Photomicrographs of crystalline deposits visualized between the teeth of a pair of Allis tissue forceps. (a) Position on forceps. (b) EDIC image. (c) Epifluorescent image of SYPRO Ruby staining. (d) Computer-rendered composite of panels b and c. Bar, 20 μm.

monitoring of cleaning standards must be maintained in order to ensure that the highest decontamination standards are reached and maintained and as such reduce any possibility of nosocomial infection.

The present investigation has looked at 260 instruments obtained anonymously from nine primary care trusts within England and Wales. They were assessed using a combination of a novel microscopy technique, sensitive fluorescent staining, and a previously described contamination index (17).

The investigation did not uncover any clear evidence of microbial or endotoxin-related bioburden. However, the high levels of proteinaceous and undefined (not positive for protein, microbial, or endotoxin) soiling were found on many of the instruments.

Interset. The interset results showed significant differences in cleaning efficacy between instrument sets obtained from different sources; however, all of the instrument sets displayed considerable amounts of proteinaceous contamination from the lowest, set 1 (CI score, 2.4), to the highest, set 5 (CI score, 3.6). This clearly indicates that cleaning efficacy is not standard over the different trusts and that in many SSDs, high levels of instrument soiling remain. None of the instruments displayed signs of microbial contamination or residual endotoxin.

Intraset. One set (set 6) contained no instruments that were defined in the protocol as being "hinged" (see Materials and Methods). The intraset findings showed that in a majority (5/8) of the sets examined, there was no significant difference between the levels of cleanliness for hinged and simple instruments. In addition, all but one set (set 4) displayed a lower CI score for the simple instruments than for the hinged instruments; this is as would be expected since the simple instruments possess fewer places for soiling to remain unaffected by cleaning. This hypothesis was confirmed by the overall results, showing a significantly lower value for the simple instruments than for the hinged group.

Intertype. The results obtained from the hinged instruments indicated that there were significant differences in soiling between the most heavily contaminated devices, needle holders and tissue forceps (CI scores, 3.8 and 3.7, respectively), and the least-soiled devices, towel clips (CI score, 3.2). It is not unexpected that towel clips should possess the lowest contamination score, due to the nature of their application, in which they are rarely in contact with the incision site or open wound. In contrast, needle holders and tissue forceps are used to aid either the suturing of or the securing of tissue away from an incision site and therefore are constantly in a position where soiling of the instrument is most likely to occur.

Of note, some of the instruments appeared heavily soiled when observed using EDIC microscopy, but this soil was not found to be proteinaceous, microbial, or endotoxin contamination positive. The soil frequently appeared crystalline in nature and may consist of deposits remaining from the use of detergent or enzymatic cleansers in the WDs. As such, this soil is a potentially hazardous material that may contaminate the patient and possibly contribute to inflammation and surgical shock. This soil would not be detected using the conventional ninhydrin or biuret protein contamination assays and may have existed as a problem for quite some time. A further potential problem associated with the crystalline deposits is the increased difficulty in removing protein compared to what was found for bare stainless steel surfaces. Clearly, improved detection methods, such as the EDIC/EF microscopy assay used here, are required to further assess the situation of nonproteinaceous soiling in SSDs worldwide and help seek improvements to WD design and operation to minimize such soiling and further improve protein removal.

In 2000, David Old chaired a review (29) of the decontamination of surgical instruments within sterile-service departments of NHS Scotland (SNHS). The Old report indicated that most of the SSD sites did not meet the published SNHS stan-

dards in a number of key areas. In another survey of Scottish SSDs, the Glennie framework (28) also indicated that in a majority of the SSDs, SNHS standards were not being met. The framework reported that only 4 of the 28 (14%) of the SSDs tested were accredited to the required EN46002 quality standard in accordance with the medical directive 93/42/EEC, and only 10% of neurosurgery and ophthalmic surgery sites met the laid down technical requirements.

In 2001, a report summarizing the findings of a comprehensive survey investigating the decontamination of surgical instruments in NHS hospitals in England and Wales was published (25). The survey assessed whether current standards were being met by all of the 249 NHS SSD units. The report categorized their establishment findings into three groups: red (standards need to be raised), amber (standards are acceptable), and green (standards are good). The initial survey found that 109 (44%) SSDs were classified as unacceptable and only 41 (16%) SSDs were classified as good. By the implementation of urgent action plans, all unacceptable hospitals had been raised to at least an amber level before the final publication of the report in December 2001. Nevertheless, still only 55 of the 249 (22%) SSDs were classified as possessing good decontamination practices. With this in mind, the Department of Health announced that an investment of £200 million would be spent on improving decontamination services in England and Wales by 2003.

The findings in the present investigation agree with those in previous surveys (25, 28, 29) and indicate that cleaning standards at the time of testing were in need of improvement. Although no evidence of microbial or endotoxin contamination was found, the extent to which there is proteinaceous and nonproteinaceous soiling must be of concern and has been linked with serious complications that may arise when instruments, even if sterile, are left within a patient (13). Either new operating procedures must be instigated, although increasing wash time within an SSD is not ideal, or new cleaning chemistries must be developed and validated. In addition, the application of presoak solutions which can both clean and maintain an instrument's wetness immediately after operative use may produce a reduction in the contaminants that an SSD is required to remove. This is a procedure that is not commonly applied at present within the NHS.

In conclusion, the present investigation gives an in situ description of proteinaceous and nonproteinaceous contamination and provides evidence that although bacteria and endotoxin are being removed effectively from surgical steel instruments, proteinaceous contamination remains. The techniques outlined allow direct visualization of bioburden, thereby negating the drawbacks inherent with traditional methods that employ soil recovery and ex situ detection techniques to assess contamination on a surface (17). The methods used in the present survey have been shown to allow sensitive quantification of the contamination and as such provide an important advance for the rapid assessment of potentially contaminated instruments.

This work may provide a major advance for public health and help to reduce iatrogenic transmission of robust infectious agents, such as the prion protein. As such, it can offer an increase in public confidence towards health care cleaning and decontamination procedures worldwide. Although it is worth bearing in mind that the ages and histories of the instruments were unknown, it is clear that that the standard of cleanliness for the surgical instruments was poor and that only with regular, controlled assessment as described in this investigation can any improvement in cleaning protocols, chemistries, and practices be judged.

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