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Research Paper

Low-level microbial contamination of liquid in syringe hubs leads to an unacceptable risk to the end product

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Abstract

Objectives The aim of this study was to assess the risk associated with microbial contamination in the hub-fluid in Luer-lock syringes to the end-product, and ultimately patients.
Methods The hub-fluid of 48 sterile syringes prefilled with broth was contaminated with a low number of *Staphylococcus epidermidis* or spores of *Bacillus subtilis*. After incubation for three weeks, the syringe fills were tested for the presence of bacterial contaminants and some syringes were used to inoculate an end product broth that was then investigated for the presence of microorganisms.
Key findings After three weeks of incubation only 20.8% of syringe fills showed turbidity, although following further investigation 70.8% were positive for the presence of viable bacteria, whereas 95.6% of end products became contaminated following injection of the syringe fill.
Conclusions These findings add quantitative data that support the current practice of discarding syringes with residue around the cap.
Keywords *Bacillus subtilis*; contamination; hub-fluid; Luer-lock syringes; *Staphylococcus epidermidis*

Introduction

Aseptic manufacturing can be described as a multiple step process by which many materials, equipments, medicinal products and containers are transferred into (and manipulated in) an environmentally controlled workspace to produce a sterile product to minimise risk of contamination to patients.[1] Sufficient quality assurance measures in pharmacy guarantee that patients are not positioned at risk of contamination or infection which may lead to fatalities.[1] Both errors in preparation and microbial contamination are often linked to syringes and infusions, with up to a third of hospital-acquired blood infections being associated with intravenous (i.v.) infusions and catheters, potentially due to inappropriate storage or contamination of critical points on the syringe.[1–3] This contamination could lead to cross contamination of injection ports, representing a significant infection risk to patients.[3,4]

A number of reasons have been identified that could be the cause of this microbial contamination; some of them include inappropriate aseptic techniques such as touching the needle or other critical points, dirty preparation areas and inappropriate storage conditions.[2] During aseptic preparation a fluid reservoir may unintentionally be created in the hub of a syringe (Figure 1) before capping and storage. This is often the case with the use of automated fillers, which are designed to fill up automatically a large number of syringes. This may provide a specific opportunity for microbiological contamination to gain access to the syringe hub and subsequently the syringe fill, and this may compromise the product integrity.[4] It has been shown that the syringe contents impact on the level of microbial contamination.[5] To date, there has been no study looking specifically at the microbiological risk associated with contamination of the syringe hub during filling. One of the current practices is to discard prefilled syringes with fluid in the hub. This is an expensive choice which is not currently backed up with scientific evidence.

This investigation aimed to determine the risk associated with a low bacterial bioburden in the hub-fluid to the contamination of prefilled Luer-lock syringes that had been stored for 21 days at room temperature and at 4°C.

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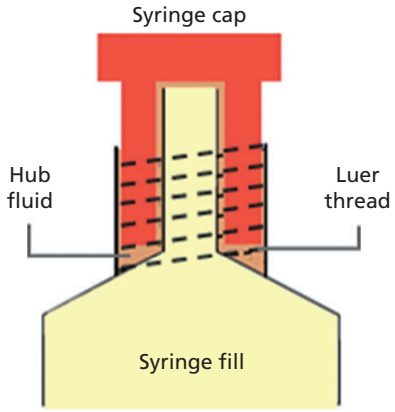

Figure 1 Schematic representation of the Luer-lock syringe, showing the hub-fluid.

Materials and Methods

Materials

Staphylococcus epidermidis NCIMB 8853 and *Bacillus subtilis* ATCC 6051 were obtained from culture collections. Growth media tryptone soy agar (TSA) and tryptone soy broth (TSB) were purchased from Oxoid Ltd (Basingstoke, UK).

Sodium chloride (NaCl) was purchased from Fisher Scientific (Loughborough, UK) and tryptone from Oxoid Ltd (Basingstoke, UK). Luer-lock syringes were purchased from Beckton-Dickinson (Oxford, UK). Sterile prefilled Luer-lock syringes containing 20 ml TSB were provided by the Sterile Products Unit at the University Hospital of Wales (Cardiff, UK).

Preparation of bacterial inocula

All stock bacterial cultures were kept as freezer stocks at -80°C in 10% glycerol. Working stock TSA slopes were stored at 4 ± 1°C. *B. subtilis* (ATCC 6051) spore stocks were prepared according to the BS EN 1276.[6] *S. epidermidis* were kept on TSA and sub-cultured to fresh TSA slopes when required. Suspensions of *S. epidermidis* were prepared by washing the TSA slope with tryptone sodium chloride (TSC; containing 1 g/l tryptone and 8.5 g/l NaCl), centrifugation at 2600*g* and resuspension in TSC. Bacterial suspensions were standardised by optical density at 500 nm and were enumerated by the drop count technique.[7] The counting method was validated on nine dilution series with a one-way analysis of variance ($P > 0.05$)) using Minitab 15 (Minitab Ltd, UK).

Inoculation of prefilled sterile syringes

Prefilled Luer-lock syringes were mounted on a stand upright and the hub region (Figure 1) was inoculated with 5 μl of a standardised culture of either *S. epidermidis* or *B. subtilis* spores containing a concentration of 100–1000 or 1000–10 000 viable bacteria or spores, using a displacement pipette. Following inoculation, syringes were capped before incubation. Inoculated syringes were stored flat at either 4 ± 1°C or at room temperature (22 ± 2°C) for three weeks inside sealed sterile autoclave bags to avoid environmental contamination. A growth control was performed whereby 5 μl standardised culture of *S. epidermidis* or *B. subtilis* spores (containing 100–1000 or 1000–10 000 viable bacteria or spores) was inoculated directly into 20 ml TSB and incubated at 37 ± 1°C under agitation. Positive growth was recorded by the presence of turbidity after 24 and 48 h. A recovery control was performed to ensure that the bacteria or spores inoculated into the hub could be recovered. A negative control was performed by inoculating the hub region of 12 syringes with 5 μl sterile TSB. The absence or presence of growth was investigated as described below.

Assessment of bacterial contamination on prefilled sterile syringes

Forty eight Luer-lock syringes with contaminated hub-fluid were used and separated equally into two groups. One group investigating hub-fluid and syringe fill contamination, and the second group investigating the transfer of syringe fill to sterile broth. Following incubation for three weeks at room temperature (22 ± 2°C) or at 4 ± 1°C, syringes were removed from storage and the presence of bacterial growth was assessed as follows:

Hub-fluid

The syringe hub-fluid was removed by aspiration and the hub rinsed with 45 μl TSC. The fluid and rinse fluid were pooled to make up the final suspension to 50 μl. Ten microlitres of this diluted suspension was spread over the surface of a TSA plate, incubated at 37 ± 1°C for 24 h and the number of colonies counted.

This protocol to recover the hub-fluid was validated in a preliminary experiment performed 10-times in triplicate to ensure that the same number of inoculated bacteria or spores was recovered following inoculation with the same inoculum concentration (data not shown).

Syringe fill

Syringe fills which appeared turbid after storage, resulting from heavy bacterial growth, were separated and recorded as a positive growth. When the fills appeared clear to the eye a sterile needle was put through the wall of the syringes. The content was drained into a sterile centrifuge tube, vortexed, passed through a 0.45-μm membrane filter (Sartorius) and placed on the surface of a TSA plate. The number of colonies was counted after 24-h incubation at 37 ± 1°C.

Potential transfer from contaminated syringes

To assess the potential transfer of bacterial contamination from prefilled syringes to end products (to mimic administration of the prefilled syringe by intravenous injection to a patient), the contents of prefilled syringes where the fill appeared clear after storage was transferred to 50 ml TSB. A positive growth was then assessed on the basis of turbidity after incubation at 37 ± 1°C for 24 h. TSB that appeared clear after incubation was investigated further for the presence of viable bacteria as described above.

Statistical analysis

The Mann–Whitney *U*-test was used to estimate the statistical significant difference of the percentage of colony forming

units (CFU) recovered between hub-fluid inoculated with a high and low inoculum in the control experiment and after storage for three weeks. $P \leq 0.05$ was considered to be statistically significant.

Results

Controls

TSB was shown to support the growth of a low number of *S. epidermidis* or spores of *B. subtilis*. All TSB bottles inoculated with 5 μl of a low inoculum (100–1000) showed turbidity after 24-h incubation at 37 ± 1°C (data not shown).

Bacteria/spores inoculated into the hub were able to be recovered after 30-min incubation. The percentage of recovery depended upon the number of bacteria/spores inoculated. The Mann–Whitney *U*-test was used to compare the statistical significance between hub-fluid inoculated with an initial high or low inoculum; the higher the number, the lower the recovery ($P \leq 0.05$, comparison between groups by Mann–Whitney *U*-test) (Table 1); this was an unexpected observation and could possibly have been caused by the presence of aggregates associated with the high inocula. This has not been confirmed in this study.

None of the syringes where hubs were inoculated with TSB showed any visible sign of contamination; i.e. no turbidity in the hub-fluid or the syringe fill, no colony recovered after incubation of membrane filter after the syringe fill was filtered and no turbidity was observed after the syringe fill was inoculated in 50 ml TSB and incubated up to 48 h at 37 ± 1°C.

Bacterial contamination in prefilled syringes after three-weeks incubation

Viable bacteria were recovered from the hub-fluid after incubation for three weeks. While the percentage recovery was low for the syringes stored at 4 ± 1°C, high numbers of bacteria were recovered from those stored at room temperature ($P \leq 0.05$, comparison between groups by Mann–Whitney *U*-test) (Table 2). In addition, there was a significant difference between the percentages of bacteria/spores recovered from an initial low inoculum when compared with an initial high inoculum; the higher the number, the lower the recovery ($P \leq 0.05$, comparison between groups by Mann–Whitney *U*-test). These results were consistent with those of the control experiment.

When contamination of the syringe fills was investigated after three-weeks storage at room temperature, only one syringe, where the hub had been inoculated with a low concentration of *S. epidermidis* (1117 CFU), showed turbidity, whereas four syringes inoculated with *B. subtilis* (i.e. two with 883 spores and two with 10 000 spores) had a contaminated fill. None of the syringes stored at 4 ± 1°C showed visible signs of turbidity. However, after extraction and filtration of the TSB from syringes showing no turbidity, signs of bacterial growth were demonstrated for all syringes that were stored at room temperature, except for one syringe inoculated with a low concentration of *B. subtilis* spore (883 spores).

When inoculated syringes were stored at 4 ± 1°C, different results were obtained (Table 3). When a low number of bacteria/spores were inoculated into the hub-fluid, 0/3 and 1/3 syringe fills were contaminated with *S. epidermidis* and *B. subtilis*, respectively. When a higher number of bacteria/spores was inoculated then 2/3 and 3/3 syringe fills showed the presence of viable *S. epidermidis* and *B. subtilis* colonies, respectively, although the number was low.

When the level of contamination of the syringe fill was considered, 66.7% of syringes with hubs inoculated with *S. epidermidis* showed some level of contamination compared with 75% of syringes inoculated with spores of *B. subtilis.*

Table 1 Recovery control of bacteria/spores inoculated into the syringe hub

Bacteria	Amount inoculated (CFU/spore in 5 μl)	Amount recovered (CFU/syringe) ± SD	Percentage recovered ± SD
Staphylococcus epidermidis	1100	792 ± 56	71.3 ± 36.0
	7416	2979 ± 181	40.2 ± 2.4
Bacillus subtilis	883	764 ± 57	86.5 ± 6.5
	10 333	3147 ± 188	30.5 ± 1.8

CFU, colony forming units. $n = 3$.

Table 2 Hub-fluid contamination after storage for three weeks at two different temperatures

Bacteria	Amount inoculated (CFU/5 μl)	Mean CFU/5 μl recovered		Percentage recovered (± SD)
Staphylococcus epidermidis	1110	22 ± 2°C	TMC	–
		4 ± 1°C	320	28.8 (± 1.4)
	7416	22 ± 2°C	TMC	–
		4 ± 1°C	510	6.9 (± 1.0)
Bacillus subtilis	883	22 ± 2°C	TMC	–
		4 ± 1°C	127	14.4 (± 4.3)
	10 000	22 ± 2°C	TMC	–
		4 ± 1°C	198	2.0 (± 0.7)

CFU, colony forming units; 22 ± 2°C, room temperature; TMC, too many to count. $n = 3$.

Table 3 Level of contamination in syringe fills following incubation for three weeks at 4°C

	Initial number inoculated (CFU/5 μl)	Syringe number	Turbidity of syringe fill	Growth on membrane filter (CFU)
Staphylococcus epidermidis	1117	1	Clear	0
		2	Clear	0
		3	Clear	0
	7416	4	Clear	0
		5	Clear	3
		6	Clear	11
Bacillus subtilis	883	7	Clear	0
		8	Clear	3
		9	Clear	0
	10 000	10	Clear	1
		11	Clear	41
		12	Clear	3

CFU, colony forming units.

Potential transfer from contaminated syringes

The inoculation of 50 ml TSB with fills from syringes with hubs inoculated with either *S. epidermidis* or *B. subtilis* resulted in heavy contamination (turbidity) after 24-h incubation at 37 ± 1°C, 7/12 TSB bottles for *S. epidermidis* and 8/11 for *B. subtilis*, respectively (one syringe inoculated with *B. subtilis* was lost during processing). Further investigation of possible contamination of TSB that showed initially no turbidity revealed that the remaining 5/12 TSB bottles contained a high number of viable *S. epidermidis* and 2/11 bottles contained a very low number of *B. subtilis* (< 2 CFU) and one was free of bacteria.

Although the number of samples was small (three per conditions tested), it appeared that the different type of bacteria and the two initial inoculum concentrations used here did not affect the contamination that occurred in the syringe fills or the transfer of contaminants in TSB. When the numbers of contaminated syringes were grouped together, 70.8% of syringe fills were contaminated and 95.6% of inoculated broth showed the presence of bacteria. However, from the direct visible assessment of turbidity only 20.8% of syringe fills showed some contamination and 70% of inoculated broth became visibly turbid.

Discussion

This study has focused on the risk associated with contaminated hub-fluid of Luer-lock syringes to final product contamination. Here the worst case scenario was investigated whereby a nutrient rich solution (TSB) was used to support the growth of bacterial contaminants and the hub was inoculated with either vegetative bacteria or bacterial spores. The two bacterial indicators used in this study, *S. epidermidis* and spores of *B. subtilis*, were realistic environmental contaminants. Although 48 syringes were used only three syringes were used per condition investigated, which limited the statistical power of this small study. However, despite this limitation, when the data were pooled, the results clearly demonstrated that the presence of low-level microbial contaminants in the hub-fluid presented a risk for the contamination of the syringe fills and to the end product following injection. This study, however, did not directly assess the risk of the hub-fluid to be contaminated by microorganisms during storage. Instead the approach taken here reflected the scenario whereby a low-level contamination occurred. Demonstrating transfer of contamination to the end product was important as this showed that contamination could be transferred from the syringe to a patient via intravenous injection of the prefilled syringe. The risk for the hub-fluid to become contaminated during storage was difficult to assess as it is multifactorial; for example place and condition of storage, and level of environmental contamination. Products to be injected intravenously or in parenteral nutrition bags are required to be sterile. Thus the risk of product contamination during storage, which might be exacerbated by the presence of hub-fluid, cannot be acceptable.

The presence of hub-fluid affected the microbiological contamination rate of syringes stored for 21 days at room temperature or at 4 ± 1°C. Even when no visible sign of contamination was observed in the syringe fill (29.2%), contamination of the end product (95.6%) occurred. In addition, one of the risks highlighted in this study was the judgement on the presence of contamination based on observation for turbidity after long-term storage. According to our observation only 20.8% of the syringe fills appeared to be turbid after three weeks. In addition, no turbid syringe fills were observed following storage at 4 ± 1°C for three weeks, whereas 50% of these syringes were positive for viable bacteria after further examination.

Although sterility testing is carried out immediately after the aseptic process, no microbiological testing of syringes is carried out immediately before administration.[8] As growth of microorganisms can be very rapid, any potential contamination reservoir in syringes stored before administration may present a significant infection risk and could be passed to the end product or patient.[9]

Revision of current aseptic technique may be required to assess this particular procedure to reduce the chance of hub-fluid formation in Luer-lock syringes during aseptic manufacture. The automation used to fill the syringes in the hospital pharmacy (such as the Rapid-Fill Automated Syringe Filler (ASF)) has been validated for its performance to produce

aseptic products. However, the mechanism designed to facilitate the filling process should be tested also and checked to assess the source of air bubble formation during the syringe-filling process, which may contribute to the generation of hub-fluid. Review of the mechanical syringe filling process could result in improvements being made by an appropriate engineer to enhance the performance of the ASFs in an attempt to eliminate syringe hub-fluid. This may help to reduce the costs associated with rejecting and discarding all the syringes presenting with hub-fluid.

Where syringes are filled manually, in cases where air bubbles are formed in the syringe fill, operators are required to tap the barrel until all air bubbles float to the top. The action of expelling the air from the syringe by pushing the plunger forward until the fluid reaches the level of the needle tip often causes the formation of hub-fluid when the operator pushes the plunger too much. The training of personnel may need to be revisited to improve the handling of Luer-lock syringes in an attempt to eliminate syringe hub-fluid.

Conclusions

Practices to deal with the presence of hub-fluid in Luer-lock syringes might differ worldwide and often follow local rules. As such they are not usually reported in the public domain. Some policies, such as the one that prompted this study, which state that when the checking procedure is carried out any syringes with residue around the cap should be rejected and discarded might be followed.[10] The consequence of such a practice is the financial loss associated with discarding syringes and products; locally in the pharmacy aseptic unit this contributes to up to a 3% loss annually (Lee Samuel, personal communication). Other policies might not make such a recommendation and where products are required to remain sterile this might be an issue. For the first time our findings add quantitative data to support such a decision. The presence of liquid in syringe hubs will be conducive and supportive of microbial growth and leads to an unacceptable risk of bacterial contamination. There may be scope for improved design of automated filler equipment used in sterile product units.

Declaration

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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