International Journal of Infectious Diseases (2009) 13, 537—542

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Mycobacterium abscessus post-injection abscesses from extrinsic contamination of multiple-dose bottles of normal saline in a rural clinic☆

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Received 15 February 2008; received in revised form 27 October 2008; accepted 8 November 2008
Corresponding Editor: Sheldon Brown, New York, USA

KEYWORDS
Mycobacterium chelonae;
Hospital infections;
Disease outbreaks;
Abscess;
Injection

Summary
Background: We investigated an outbreak of gluteal abscesses following intramuscular (IM) injections given at a clinic in rural China to identify the causative agent, source, and method of exposure.
Methods: We defined a case as an abscess that appeared at the site of an injection given since June 1, 2006. We compared case rates by injection route, medication, and diluents. We reviewed injection practices, and cultured abscesses and environmental sites for mycobacteria.
Results: From October through December 2006, 5.8% ($n = 35$) of 604 persons who had received injections at the clinic developed a case. All 35 cases occurred in 184 patients (attack rate = 19.0%) who had received IM injections with various drugs that had been mixed with normal saline (NS); risk ratio = ∞; $p < 0.0001$. No cases occurred in the absence of NS exposure. We identified *Mycobacterium abscessus* from eight abscesses and from the clinic water supply, and observed the inappropriate reuse of a 16-gauge needle left in the rubber septum of 100 ml multiple-dose bottles of NS in the clinic. Fourteen percent ($n = 527$) of the 3887 registered residents of this village had been treated with IM drugs over a three-month period, often for minor illnesses.
Conclusions: This outbreak of *M. abscessus* occurred from exposure to extrinsically contaminated NS through improper injection practices. Frequent treatment of minor illnesses with IM

DOI of original article: 10.1016/j.ijid.2008.11.025.
☆ Presented in part at the 56th Epidemic Intelligence Service (EIS) Conference, Atlanta, Georgia, USA, April 16—20, 2007.
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doi:10.1016/j.ijid.2008.11.024

injections of antibiotics was likely an important contributing factor to the size of this outbreak.

Introduction

Injection safety is an enormous global health challenge both in the developing world, where safe injection practices are often lacking, and in developed countries, where new technologies and the transition of clinical practice to less regulated outpatient settings is occurring.[1–3] *Mycobacterium abscessus* and related rapid-growing mycobacteria (RGM) have caused iatrogenic post-injection abscesses as well as a variety of skin and soft tissue infections in these settings.[4–7] Outbreaks of post-injection abscesses from RGM usually result from contamination of injectable solutions or injection equipment in the immediate healthcare setting.[7] They are particularly problematic in that antibiotic treatment is often delayed because RGM are often resistant to multiple antibiotics and require special culture media.[7] In addition to the need for vigilance to interrupt transmission from these extrinsic local sources, there is the concern of intrinsic contamination of a commercially distributed product,[8] with epidemic potential. Therefore, all clusters or outbreaks of these abscesses demand prompt investigation.

In December 2006, we received a request to investigate why abscesses had developed at sites of drug injections given at a rural clinic (clinic Y) serving village Y in Guangdong Province, China. Acid-fast bacilli (AFB) were identified from four of five abscesses. Initial interviews and a review of medical records indicated that all the patients had received cefradine, ribostamycin, dexamethasone, or ribavirin injections. Suspicion was cast on these medications and on normal saline (NS). We began an investigation to identify the causative agent, source, and method of acquiring these abscesses.

Methods

Case finding

We defined a case as an abscess or persistent induration at the site of any injection given between June 1, 2006, and February 1, 2007, at any of the four village clinics affiliated with one township hospital. We found cases by searching medical records in all four village clinics, follow-up of patients who had received intramuscular (IM) and/or intravenous (IV) injections at clinic Y, and a house-to-house search of village Y. Physicians at the local township hospital had performed incision and drainage of abscesses of patients at the time of presentation for clinical care. They had submitted samples of pus to the clinical laboratory that served their hospital. We obtained AFB isolates from the clinical laboratory, determined their antibiotic sensitivities, and identified them using standard biochemical techniques.

Retrospective cohort study

To identify which drug, diluent, or instrument was the source of this outbreak, we identified all orders for IM or IV injections at clinic Y from August to October 2006. From these we determined the patient, medication, diluent, and route of each injection. We then compared rates of abscess incidence for each of these exposures.

Evaluation of injection practices and clinic environment

We reviewed injection procedures in the treatment room. We ascertained the manufacturer and lot numbers of all injectable drugs and the source of other components of each injection. Using standard environmental sampling methods,[9,10] we took environmental specimens of air, materials, surfaces, and water (including well water supply) to culture for mycobacteria.

We undertook this investigation to respond to an acute problem of adverse events. The principal objective was to identify the mode of exposure and source of infection to terminate the outbreak and benefit the affected community. Responses of this nature are not considered research and accordingly do not require institutional review board approval. Moreover, we performed no additional procedures, tests, data collection, or data analysis above those needed to resolve the immediate public health problem.

Results

Case finding and characteristics

We identified 35 cases, including 33 in residents of village Y and two in residents of a neighboring village. All 35 had received injections from clinic Y. The onset of first noticeable symptoms was from October 14, 2006 through January 22, 2007. The antecedent injections had been given from August to October 2006. Ten cases occurred in males and 25 in females. The ages of patients ranged from 8 months to 96 years. All 35 had abscesses and 69% ($n = 24$) also had persistent induration at the injection site. Excluding two patients with upper respiratory symptoms, 21% ($n = 7$) of patients also had fever from $>37\ ^\circ C$ to $38.5\ ^\circ C$. Twenty-six percent ($n = 9$) had inguinal lymphadenopathy. One case patient had abscesses in both buttocks and the rest had abscesses in either the left (49%; $n = 17$) or right (49%; $n = 17$) buttock. Some case patients reported pain or hyperalgesia at the injection site. Chest films were clear for all case patients. Twenty-eight cases were treated by incision and drainage and one by needle aspiration. Two abscesses drained spontaneously. Four patients did not receive treatment. Pus from eight of 26 abscesses yielded *M. abscessus*. All eight isolates were resistant to isoniazid, rifampin, *p*-aminosalicylic acid, levofloxacin, and capreomycin. They were sensitive to clarithromycin, amikacin, and ethambutol.

Retrospective cohort study

During the period of the cohort analysis, August through October 2006, 838 injections were given to 604 patients at

Table 1 Exposures of 35 abscess case patients among 604 patients exposed to 838 injections from clinic Y by drug and route of injection; Guangdong Province, China, August—October 2006

Injection type and drug[a]	Exposed		Unexposed		% Exposed	Attack rate (%)		RR	95% CI
	Case	Total	Case	Total		Exposed	Unexposed		
Injection route									
Any IM	35	527	0	77	87	6.6	0	∞	-
Any IV	5	153	30	451	25	3.3	6.7	0.49	0.19—1.2
Only IV	0	77	35	527	13	0	6.6	0	-
IM injections									
Any with NS	35	184	0	420	30	19.0	0	∞	-
Any without NS	16	422	19	182	70	3.8	10.4	0.36	0.19—0.69
Only without NS	0	343	35	261	57	0	13.4	0	-
IM with NS in syringe by drug[b]									
Ribostamycin	16	67	19	117	36	23.8	16.2	1.5	0.81—2.7
Cefradine	24	129	11	55	70	18.6	20	0.93	0.49—1.8
Ribavirin	8	45	27	139	24	17.8	19.4	0.92	0.45—1.9
Dexamethasone	26	140	9	44	76	18.6	20.5	0.91	0.46—1.8
IM without NS in syringe by drug									
Dexamethasone	0	82	35	522	14	0	6.7	0	-
Ribavirin	0	7	35	597	1.2	0	5.9	0	-

RR, risk ratio; CI, confidence interval; IM, intramuscular injection; IV, intravenous injection; NS, normal saline.
[a] Patients may have been exposed to more than one injection type and drug.
[b] $N = 184$ patients.

clinic Y. Of these patients 5.8% ($n = 35$) developed a case. All 35 cases occurred in 184 patients (attack rate = 19.0%) who had received IM injections with one or more of four different drugs (cefradine, ribostamycin, dexamethasone, and ribavirin) dissolved in NS (Table 1). Two of these drugs, dexamethasone and ribavirin, did not require reconstitution in NS but were sometimes mixed in the same syringe with drugs needing reconstitution in NS (cefradine and ribostamycin). When given in this combination, dexamethasone and ribavirin were associated with an increased risk of abscesses. In contrast, when given without NS, they had zero risk. Among the 184 patients given IM injections with NS, none of the four individual medications was associated with a greater risk of abscesses. Finally, the risk of having an abscess increased as the number of NS-containing injections increased ($p < 0.05$; Chi-square for trend = 6.1). No patient receiving an injection without NS developed a case.

To better define the period of exposure we calculated a case-injection/total injection rate in intervals of one-third month. We also identified 18 case patients who had received only one intramuscular injection. The case-injection/total injection rate rose sharply from a baseline during the middle of August (Figure 1). All cases had had one or more IM injections containing NS between August and October 2006. Injections of case patients who had received only one injection began to appear in mid-August. The clinic began to use 100 ml bottles of NS in June when it ran out of 2 ml vials of NS. Injections with NS were stopped on October 23, 2006. Using the nine cases with single injections who could report an onset day, we estimated the median incubation period to be 31 days (range 13—87 days).

Assessment of injection practices and clinic environment

Beginning in June 2006, the nurse responsible for giving injections at the clinic began using multiple-dose bottles (100 ml) of NS (Figure 2) to dilute cefradine and ribostamycin. The nurse routinely left a 16-gauge needle inserted through the septum of the bottle. When the bottle was not in use the nurse attached a syringe to the hub of this needle. To prepare an injection the nurse would detach the syringe from this needle, lay the syringe on the table and attach another syringe to be used for injection to the needle and withdrew NS. She would use this second syringe and NS to dilute the drug. She would then aspirate drugs into the second syringe, give the injection, and reattach the first syringe to the needle in the saline bottle. The nurse stated

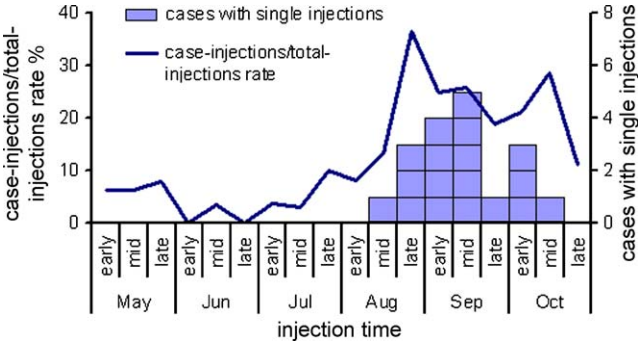

Figure 1 Intramuscular injections with normal saline by date, with rate for all cases and count for cases with only one injection; clinic Y, Guangdong Province, China, August—October 2006.

Figure 2 Normal saline bottle showing the open 16-gauge needle and syringe used to plug the needle hub.

that she changed the syringe used to plug the needle hub in the NS bottle whenever she began using a new bottle of NS, about every three to seven days. She never used disinfectant to clean the septum of the NS bottle. To prepare the skin for injection the nurse took cotton balls directly from original bags and wet them with 75% ethanol. The clinic stopped using 100 ml NS for IM injections on October 23, 2006 and stopped cefradine or ribostamycin injections on November 13, 2006. The nurse used the same lot of NS from June through October.

Environmental samples taken in December revealed *M. abscessus* from the clinic well water. The well water was not chlorinated or otherwise treated or disinfected. We found AFB from the top of the cabinet for storing syringes, and the sewage from the clinic, but these were not *M. abscessus*. Sampling of 37 other sites including the injection table, the cloth used to wipe down the table, the sink and drain, the antiseptic solution, air, syringes, and needles did not yield AFB. The nurse's hands were negative for AFB and she had no pulmonary disease.

Two of the three other clinics served by the township hospital used NS from the same lot number and from the same manufacturer. From August to December 2006 these two clinics attended to 2649 outpatients and used 240 bottles of the same lot of NS to prepare injections. The nurses in these two clinics used the same technique for preparing drugs for injection as the nurse at clinic Y with one difference. They opened a new bottle of NS each day. We identified no post-injection abscesses among patients of those two clinics.

Village Y had a population of 3887 registered residents. Using this population, we can estimate from our cohort data that 14% (n = 527) received IM and 4% (n = 153) received IV drugs over the three months covered by our investigation. The clinic physician reported that he used parenteral drugs to treat minor illnesses as he referred patients with more severe illnesses directly to the township hospital.

Discussion

We concluded that this outbreak of *M. abscessus* abscesses occurred due to an unsafe injection practice, specifically the reuse of syringes on 100 ml multiple-dose bottles of NS. Outbreaks of post-injection abscesses from RGM, including *M. abscessus*, have resulted from both intrinsic and extrinsic contamination of injectable drug preparations or injection equipment.[8,11–14] In this investigation concerns about the quality of the injectable drugs and the NS required that we quickly distinguish between intrinsic and extrinsic contamination. Several lines of evidence led us to the conclusion that extrinsic contamination from the clinic was responsible. These include the specific association with NS, exposure dates beginning after one month of using the NS, the lack of abscesses in other clinics that used the same NS, the lack of association with any specific drug used with NS, the risk ratio of zero for ribavirin and dexamethasone when they were not combined with NS, and management of the multiple-dose NS bottle that would allow contamination and time for growth of *M. abscessus* in the NS. Finding *M. abscessus* in the clinic water supply also supports this conclusion. In other health-care related outbreaks of *M. abscessus* and other RGM, the organism has also been found in the tap water supply.[15,16] *M. abscessus* is an aquatic organism that can propagate in water or soil and can even grow in distilled water.[17] Thus, finding it in the clinic well is not highly specific, since it could be found in any hospital or clinic water supply.[18] Moreover, we could not confirm that *M. abscessus* was present in the water at the time of the exposure or determine how it was transferred from the tap water to the NS.

The pattern of exposure days indicates that several bottles of NS were used without incident from June through early August, followed by more than nine consecutive contaminated NS bottles. This pattern would be unlikely if the contamination had occurred sporadically from contamination of the hub of the needle from airborne *M. abscessus* or if the nipple of the syringe had picked up *M. abscessus* from the tabletop or air. More likely, in early August one NS bottle was contaminated via the open needle. Since *M. abscessus* can multiply in water,[17] this initial contamination would have led to a more heavily contaminated bottle. Subsequent bottles were probably contaminated if the nurse used the syringe from a contaminated bottle to plug the needle of a new bottle. Although the nurse denied doing this, we could not postulate another mechanism for the serial contamination. In the 11 previously reported outbreaks of post-injection RGM abscesses, the source of the organism has been identified in three.[8,13,14] In two of these three the source was water and in one it was soiling of the top of penicillin vials from a damp storage area.

Underlying this outbreak, one can detect an important contributing factor, the frequent use of injections of antibacterial and antiviral agents. Indeed, a recent outbreak of abscesses from a related RGM (*Mycobacterium massiliense*) followed injection with ribostamycin for minor respiratory

infections.[11] Patients in our outbreak often received only a single injection of drugs that actually required multiple doses over several days to be effective in treating any infection. Although we did not explore the reasons for giving so many ineffective injections, this observation is not unusual. Other studies in poor, rural areas of China have revealed that 37—45% of patients who consulted rural, township hospitals received injections as treatment.[19,20] These injection rates in these studies and our investigation greatly exceed the 3.7% average estimated for transitional and developing countries.[21]

Alternative outpatient treatments have contributed to a larger number of incidents of post-injection abscesses from RGM. Mesotherapy, which consists of soft tissue injection of a variety of substances with local anesthetics, is popular in France and Latin America. It has caused outbreaks and individual cases.[12,22—24] Tissue augmentation, consisting of injections of presumably inert substances by illicit practitioners, has caused breast and facial abscesses.[25,26] Outbreaks of post-injection abscesses have also resulted from a jet injector used in a podiatry clinic and an unregistered commercially distributed product containing hydrocortisone.[8,13]

The practice of leaving needles in multiple-dose vials was modeled to all of the nurses in the four village clinics by the chief nurse in the township hospital. Chinese hospital infection management guidelines cover reuse of syringes and needles and prohibit the reuse of a multiple-dose bottle for more than 24 hours for dilution of drugs.[27] World Health Organization guidelines on safe injections state categorically that the practice of leaving a needle or syringe and needle inserted continuously through the injection septum of a bottle is dangerous and should not be done.[21] In addition to halting this practice locally, we also trained and required staff to follow correct injection techniques using official guidelines and to assure a safe water supply to the health services. Indeed, local efforts to improve injection practices can be effective.[28] Efforts to reduce the frequency of inappropriate use of injections may also be effective in preventing post-injection infections in China and elsewhere.[28,29] Such efforts would not only be expected to reduce infections, but save costs as well.[30]

Acknowledgements

I would like to thank the technician Xinshan Cai, from the thoracic hospital of Guangzhou, Guangdong Province, for laboratory support.

Conflict of interest: No conflict of interest to declare.

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